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(54) Title: METHODS OF DIAGNOSIS OF AUTOIMMUNE DISEASE

(57) Abstract: Methods of diagnosis of autoimmune disease or of a predisposition or susceptibility to autoimmune disease by detecting a polymorphism in the ESE-1, ESE-2 or ESE-3 gene. Methods of therapy of autoimmune disease in individuals having a polymorphism in the ESE-1, ESE-2 or ESE-3 gene.

METHODS OF DIAGNOSIS OF AUTOIMMUNE DISEASE

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/376,139, filed April 26, 2002 and U.S. Provisional Application No. 60/329,158, filed October 12, 2001. The entire teachings of the above applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Autoimmune disease is caused when one's own immune system incorrectly attacks one's own tissue. One important unmet medical need is the prevention of the development of autoimmune diseases such as diabetes and multiple sclerosis. At the moment, these diseases cannot be prevented and treatment can only occur after the disease has developed and it is too late to prevent the damage done to the tissue or organ, because there are no good predictive markers for diabetes and other autoimmune diseases which could be used for predictive purposes and eventually for early intervention prior to disease development.

The major shortcomings of the currently available antiinflammatory drugs is that they all have significant side effects, including gastrointestinal irritation and bleeding, bone loss, and fluid retention, some of which can be life-threatening. Other drugs that target only a single gene involved in inflammatory processes are not effective enough, since only a single component of inflammation is targeted leaving all the other components untouched.

Thus, it would be of particular advantage to be able to identify those individuals having a predisposition or increased susceptibility to autoimmune disease. Preventive therapy to reduce or prevent or delay onset of autoimmune disease could then be put into place.

SUMMARY OF THE INVENTION

The present invention relates to methods of diagnosing autoimmune disease (e.g., diabetes, multiple sclerosis) or of a predisposition or susceptibility to autoimmune disease by detecting a polymorphism in the ESE-1, ESE-2 or ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by the ESE-1, ESE-2 or ESE-3 gene, respectively. Detection of a polymorphism in the ESE-1, ESE-2 or ESE-3 gene is indicative of the occurrence of autoimmune disease or a predisposition or susceptibility to autoimmune disease.

The invention also relates to methods of treating autoimmune disease in an individual comprising screening an individual for a genetic predisposition to autoimmune disease by detecting the presence of a polymorphism in the ESE-1, ESE-2 or ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by the ESE-1, ESE-2 or ESE-3 gene, respectively, and if such a predisposition is identified, treating the individual to prevent or reduce autoimmune disease or to delay the onset of autoimmune disease.

The invention further relates to methods of treating autoimmune disease in an individual having a polymorphism in the ESE-1, ESE-2 or ESE-3 gene which is correlated with an alteration in the activity or expression of the ESE-1, ESE-2 or ESE-3 gene, respectively, by administering to the individual an agent that interferes with or blocks the function or expression of the ESE-1, ESE-2 or ESE-3 gene, respectively.

The invention also relates to treating or preventing inflammation in an individual having a polymorphism in the ESE-1, ESE-2 or ESE-3 gene which is correlated with an alteration in the activity or expression of the ESE-1, ESE-2 or ESE-3 gene, respectively, by administering to the individual an agent that interferes with, blocks or enhances the function or expression of the ESE-1, ESE-2 or ESE-3 gene, respectively.

The invention also relates to methods for assaying a sample for the presence of a polymorphism in the ESE-1, ESE-2 or ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by the ESE-1, ESE-2 or ESE-3 gene, respectively, by use of an antibody or nucleotide sequence

that specifically binds to the ESE-1, ESE-2 or ESE-3 gene, respectively, or the polypeptide encoded by the ESE-1, ESE-2 or ESE-3 gene, respectively.

In a particular embodiment, the polymorphism is a SNP at position -140 in the ESE-3 gene, and which is a guanine (G) nucleotide in comparison to an adenine (A) nucleotide in the wildtype ESE-3 gene. In another embodiment, the polymorphism is a SNP at position -4458 in the ESE-3 gene, and which is a thymine (T) nucleotide in comparison to a cytosine (C) nucleotide in the wildtype ESE-3 gene. In a third embodiment, the polymorphism is a SNP at position +171 in the ESE-1 gene, and which is a cytosine (C) nucleotide in comparison to a thymine (T) nucleotide in the wildtype ESE-1 gene. In a fourth embodiment, the polymorphism is a SNP at position +949 in the ESE-1 gene, and which is a thymine (T) nucleotide in comparison to a guanine (G) nucleotide in the wildtype ESE-1 gene. In a fifth embodiment, the polymorphism is a SNP at position +1275 in the ESE-1 gene, and which is an adenine (A) nucleotide in comparison to a cytosine (C) nucleotide in the wildtype ESE-1 gene. In a sixth embodiment, the polymorphism is a SNP at position +1639 in the ESE-1 gene, and which is an adenine (A) nucleotide in comparison to a guanine (G) nucleotide in the wildtype ESE-1 gene. In a seventh embodiment, the polymorphism is a SNP at position +1744 in the ESE-1 gene, and which is an adenine (A) nucleotide in comparison to a guanine (G) nucleotide in the wildtype ESE-1 gene. In an eighth embodiment, the polymorphism is a SNP at position -2034 in the ESE-1 gene, amd which is a cytosine (C) nucleotide in comparison to a adenine (A) nucleotide in the wildtype ESE-1 gene. In a ninth embodiment, the polymorphism is a SNP at position -151 in the ESE-2 gene, and which is a cytosine (C) nucleotide in comparison to a guanine (G) nucleotide in the wildtype ESE-2 gene. Other sequence polymorphisms in the ESE-1, ESE-2 and ESE-3 genes are provided in Figure 1B and additional embodiments of the invention relate to these sequence polymorphisms (SNPs).

Autoimmune diseases include diabetes (e.g., Type I or insulin-dependent diabetes (IDD), Type II or non-insulin dependent diabetes (NIDD)), multiple sclerosis (MS), rheumatoid arthritis (RA), lupus, psoriasis, asthma, myasthenia gravis, Sjogrens syndrome, Hashimoto's thyroiditis and Pemphigus vulgaris.

The invention also extends to products useful for carrying out the methods, such as diagnostic kits.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show the SNPs of potential functional significance in diabetes and other autoimmune diseases. Figure 1A includes SEQ ID NOs:1-14, numbered sequentially from top to bottom of the left column (SEQ ID NOs:1-8) and continuing sequentially from top to bottom of the right column (SEQ ID NOs:9-14). Figure 1B includes SEQ ID NOs:15-55, numbered sequentially from top to bottom.

Figure 2 shows the ESE-3 promoter SNP associated with Type I diabetes (SEQ ID NO:56).

Figures 3A-3D show the distribution of SNPs in the TNF-α, lymphotoxin (LT), ESE-3 and CTLA4 genes in Type I diabetes patients, Type II diabetes patients and control patients. Figure 3D also shows the results of a SNP analysis of the SNP distributions shown in Figures 3A-3D for the TNF-α, LT, ESE-3 and CTLA4 genes. The SNP analysis shows the percentage of SNPs relative to wildtype in Type I diabetes and Type II diabetes patients.

Figures 4A and 4B show the results of a statistical analysis of the SNP associations with Type I diabetes (IDD) relative to control for the SNP distributions shown in Figures 3A-3D. The statistical analysis shows the probability (p values) that the data are significant. The closer the p value is to 0, the higher the probability that the data is highly relevant.

Figures 5A and 5B show the results of a statistical analysis of the SNP associations with Type II diabetes (NIDD) relative to control for the SNP distributions shown in Figures 3A-3D.

Figures 6A-6E show the distribution of SNPs in the TNF-α, LT, ESE-3, CTLA4, TGF-β and IL-6P genes in Type I diabetes patients (IDD), Type II diabetes (NIDD) patients and control patients.

Figure 7 shows the results of a SNP analysis in diabetes patients of the SNP distributions shown in Figures 6A-6E for the TNF-α, LT, ESE-3, CTLA4, TGF-β

and IL-6P genes. The SNP analysis shows the percentage of SNPs relative to wildtype in IDD and NIDD patients.

Figure 8 shows the results of a statistical analysis of SNP associations with IDD and NIDD for the SNP distributions shown in Figures 6A-6E. The statistical analysis shows the probability (p values) that the data are significant.

Figures 9A-9C show the distribution of SNPs in ESE-3 in multiple sclerosis (MS) patients and control patients.

Figure 10 shows the results of a SNP analysis in MS patients of the SNP distributions shown in Figures 9A-9C. The SNP analysis shows the percentage of SNPs relative to wildtype in MS patients.

Figure 11A shows the results of an experiment assessing ESE-3 expression in response to IL-1β stimulation in human synovial fibroblasts. The results show that ESE-1 expression can be induced by IL-1 in synovial fibroblasts.

Figure 11B shows the results of an experiment assessing ESE-3 expression in response to IL-1β, TNF-α or IFN-γ stimulation in human chondrocyte cell lines T/C28a2, T/C28I2 and C20A4.

Figure 11C shows the results of a time course experiment examining the kinetics of ESE-3 induction by IL-1β, TNF-α or IFN-γ in the human chondrocyte cell line T/C28a2.

Figure 11D shows the results of an experiment examining the effects of lipopolysaccharide (LPS) on ESE-3 expression in monocytes using the human monocytic cell line THP-1.

Figure 12A shows the results of experiments examining the transcriptional activation of the ESE-3 promoter by IL-1β and LPS. T/C28a2 chondrocyte cells, RAW cells, HSG and U-138 cells were transfected with a pXP2/ESE-3 promoter construct and incubated in the absence or presence of IL-1β or LPS.

Figure 12B shows the results of experiments assessing the ability of NF-κB/rel family members p50 and p65 to interact with the NK-κB binding site in the ESE-3 promoter. RAW cells were cotransfected with either the parental pXP2 luciferase plasmid or a ESE-3 promoter/pXP2 luciferase construct together with p65, p50 or empty vector.

Figure 13 shows the results of an experiment assessing the ability of NF-κB/rel family members p50 and p65 to interact with the NF-κB binding site in the ESE-3 promoter in whole cell extracts from unstimulated and IL-1β stimulated U-138 MG cells. The labeled human ESE-3/NF-κB oligonucleotide probe were carried out with either no antibody or antibodies against p50, p65, relB, p52, c-rel and bcl-3. The arrow indicates the NF-κB DNA-protein complex.

Figure 14 shows the results of an experiment examining the effect of a mutation in the ESE-3 promoter at the NK-κB binding site on ESE-3 induction by LPS. The results show that mutation of the NK-κB binding site within the ESE-3 promoter abolishes induction by LPS.

DETAILED DESCRIPTION OF THE INVENTION

A SNP (Single Nucleotide Polymorphism) is a difference for one base compared with the sequence found in the majority of the studied population. This polymorphism can lead to a change in the amino acid composition of the resulting polypeptide. Even when the SNP does not lead to a change in the amino acid, there may be consequences on the level of production or protein identity, such as in case of a SNP affecting the editing of the RNA.

A SNP can be detected using the Nanogen® technology. First, PCR is carried out on genomic DNA using primers on both side of the suspected SNP position. Second, sample is loaded on an array. Using the charge properties of the DNA, the PCR products are addressed one after the other onto a microchip containing electronically controllable micro-pads. Third, a SNP is detected by hybridization of fluorescent primers. The array is subsequently flooded with a solution containing a mixture of two short oligonucleotides. Each oligonucleotide is labeled with a Cy3 or Cy5 and can hybridize specifically with a particular genotype. Scanning for color on each pad is performed after progressive stringency increase by means of temperature and washings. The ratio of fluorescence reflects the presence of each allele.

The present invention relates to Applicants' discovery that ESE-3 is a critical mediator of inflammation in non-epithelial cells. ESE-3 expression is rapidly and transiently induced in several cell types in response to inflammatory stimuli such as

interleukin-1 (IL-1), tumor necrosis factor alpha (TNFα) and endotoxin, including endothelial cells, vascular smooth muscle cells, monocytes, synovial fibroblasts, osteoblasts, glial cells and chondrocytes. ESE-3 is not expressed in these cells prior to stimulation with inflammatory stimuli. This process requires activation of the NF-κB p50 and p65 family members which induce ESE-3 expression via a high affinity NF-κB binding site within the ESE-3 promoter. The results described herein indicate that ESE-3 may be a master switch for turning on a certain set of genes that are required for inflammation. As such, ESE-3 is an ideal target to treat inflammatory processes in its earliest stages.

The present invention further relates to Applicants' discovery of the ESE-3 gene as a genetic locus that is associated with diabetes, multiple sclerosis and other autoimmune diseases. The DNA is used as a diagnostic or prognostic marker for genetic predisposition to diabetes, multiple sclerosis, and other autoimmune diseases.

SNPs within the ESE-3 gene may be involved in diabetes and other autoimmune diseases (Figures 1A and 1B). For example, the results described herein reveal that a SNP in the promoter region of the ESE-3 gene is strongly associated with Type I diabetes and likely associated with multiple sclerosis and other autoimmune diseases. Accordingly, the SNP in the ESE-3 promoter is a valuable diagnostic and prognostic marker for genetic predisposition to Type I diabetes, multiple sclerosis and other autoimmune diseases and an ideal and highly specific target for drugs. The results described herein also reveal a SNP in the ESE-3 gene that is likely associated with Type II diabetes and may be associated with other autoimmune diseases. Accordingly, this SNP is a valuable diagnostic and prognostic marker for genetic predisposition to Type II diabetes and other autoimmune diseases and an ideal and highly specific target for drugs.

Patients were analyzed for the presence of SNPs in the TNF-α, lymphotoxin (LT), ESE-3, CTLA-4, TGFβ and IL-6P genes using the Nanogen Microelectronic Chip System (Figures 3A-3D, 4A-4B, 5A-5B, Figures 6A-6E and 7-8). Sixty-two Type I diabetes (IDD), 39 Type II diabetes (NIDD) and 48 control patient genomic DNAs were initially used for this analysis (Figures 3A-3D, 4A-4B and 5A-5B).

Subsequently, an additional 42 IDD, 36 NIDD and 48 control patient genomic DNAs were included in this analysis (Figures 6A-6E and 7-8). An ESE-3 SNP was identified in the promoter region of the ESE-3 gene (-140) (Figures 1A, 1B and 2). Significant differences in SNP ratios were found for both the lymphoxin and the ESE-3 gene polymorphisms in Type I, but not Type II, diabetes patients. Both the lymphotoxin and this ESE-3 SNP were significantly elevated in Type I diabetes patients when compared to controls (Figure 1A and 1B). The percentage of Type I diabetes patients harboring this ESE-3 SNP was 44% as compared to 28% in the controls and 27% in Type II diabetes patients (Figures 3A-3D, 4A-4B and 5A-5B). These data demonstrate a strong linkage between this ESE-3 SNP and Type I diabetes. Other data described herein indicate that this ESE-3 SNP (-140) linked to Type I diabetes is likely also linked to MS. Eighty-seven MS patient genomic DNAs along with 30 control patient genomic DNAs were used for this analysis (Figures 9A-9C and 10). The percentage of MS patients harboring the ESE-3 SNP was 41% as compared to 24% in the controls (Figures 9A-9C and 10).

Another ESE-3 SNP was also identified in the ESE-3 gene (-4458) (Figures 6A-6E and 7-8). The data herein suggest that this ESE-3 SNP (-4458) may be linked to Type II diabetes.

Since SNPs in the ESE-3 gene have been directly linked to asthma patients, the data suggest that polymorphisms in the ESE-3 gene may be linked to various diseases involving the immune system such as diabetes (e.g., Type I diabetes, Type II diabetes), multiple sclerosis, rheumatoid arthritis, lupus, psoriasis, asthma, myasthenia gravis, Sjogrens syndrome, Hashimoto's thyroiditis and Pemphigus vulgaris.

The ESE-3 SNP located in the 5' upstream promoter region of the ESE-3 gene may alter transcription by changing affinity or specificity of a distinct transcription factor leading to decreased or increased ESE-3 expression in patients predisposed to diabetes, MS or other autoimmune diseases.

ESE-1, ESE-2 and ESE-3 are all members of the ESE branch of the ETS transcription factor family expressed in epithelial cells and have high sequence homology. Thus, the results described herein for ESE-3 are expected to be similar

for ESE-1 and ESE-2. In addition, ESE-1 is similar to ESE-3 as both can be activated by the proinflammatory compounds IL-1β, TNF-α and bacterial LPS in non-epithelial and epithelial cells. ESE-1 induction by IL-1β, TNF-α and bacterial LPS, similar to ESE-3 induction by IL-1β, TNF-α and bacterial LPS, is controlled via the NF-κB pathway. NF-κB p50 and p65 family members bind to a NF-κB site within the ESE-1 promoter to induce ESE-1 expression, while NF-κB p50 and p65 family members bind to a NF-κB site within the ESE-3 promoter to induce ESE-3 expression. Further, ESE-2 and ESE-3 genes are adjacent to each other on human chromosome 11 and since SNPs come as haplotypes, it is expected that a haplotype in ESE-2 includes SNPs in ESE-3 and a haplotype in ESE-3 includes SNPs in ESE-2. ESE-1 is also referred to as ESX, ELF3, ERT and JEN. ESE-2 is also known as ELF5. ESE-3 is also referred to as EHF.

Three highly related human genes, *ESE-1*, *ESE-2* and *ESE-3*, associated with a genetic predisposition to diabetes, MS and other autoimmune diseases are provided. Figures 1A, 1B and 2 show SNPs within the ESE-1, ESE-2 and ESE-3 genes of potential functional significance in diabetes and other autoimmune diseases. Nucleic acid compositions and antibodies specific for the ESE-1, ESE-2 and ESE-3 proteins are useful as diagnostics to identify a hereditary predisposition to diabetes, MS and other autoimmune diseases.

Diagnosis of ESE-1, ESE-2 and ESE-3 linkage to diabetes (Type I or Type II) and other autoimmune diseases is performed by protein, DNA or RNA sequence and/or hybridization analysis of any convenient sample from a patient, e.g. biopsy material, blood sample, scrapings from cheek, etc. A nucleic acid sample from a patient having diabetes or other autoimmune disease that may be associated with ESE-1, ESE-2 and ESE-3, is analyzed for the presence of a predisposing polymorphism in the ESE-1, ESE-2 or ESE-3 gene, respectively. A typical patient genotype will have at least one predisposing mutation on at least one chromosome. The presence of a polymorphic *ESE-1*, *ESE-2* or *ESE-3* sequence that affects the activity or expression of the gene product, and confers an increased susceptibility to diabetes or other autoimmune diseases is considered a predisposing polymorphism. Individuals are screened by analyzing their DNA or mRNA for the presence of a

predisposing polymorphism, as compared to a diabetes or other autoimmune disease neutral sequence. Specific sequences of interest include any polymorphism that leads to diabetes or other autoimmune diseases or is otherwise associated with diabetes or other autoimmune diseases, including, but not limited to, insertions, substitutions and deletions in the coding region sequence, 3' and 5' untranslated regions that affect RNA stability or transcription, intron sequences that affect splicing, or promoter or enhancer sequences that affect the activity and expression of the protein. Examples of a specific *ESE-3* polymorphism in Type I diabetes patients are listed in Figures 2, 3A-3D and 6A-6E. Examples of a specific *ESE-3* polymorphism in Type II diabetes patients are listed in Figures 6A-6E. Examples of a specific *ESE-3* polymorphism in MS patients are listed in Figures 9A-9C. Other specific sequence polymorphisms in the ESE-1, ESE-2 and ESE-3 genes are provided in Figures 1A and 1B.

The effect of an *ESE-1*, *ESE-2* or *ESE-3* predisposing polymorphism may be modulated by the patient genotype in other genes related to diabetes and other autoimmune diseases, including, but not limited to the lymphotoxin, TNF-α, CTLA-4, IL-10, IL-6P, TGF-β, Fc.epsilon receptor, Class I and Class II HLA antigens, T cell receptor and immunoglobulin genes, cytokines and cytokine receptors, chemokines and chemokine receptors and the like.

Screening may also be based on the functional or antigenic characteristics of the protein. Immunoassays designed to detect predisposing polymorphisms in ESE-1, ESE-2 or ESE-3 proteins may be used in screening.

Biochemical studies may be performed to determine whether a candidate sequence polymorphism in the *ESE-1*, *ESE-2* or *ESE-3* coding regions or control regions is associated with disease. For example, a change in the promoter or enhancer sequence that affects expression of *ESE-3* may result in predisposition to Type I diabetes. Expression levels of a candidate variant allele are compared to expression levels of the normal allele by various methods known in the art.

Methods for determining promoter or enhancer strength include quantitation of the expressed natural protein; insertion of the variant control element into a vector with a reporter gene such as β-galactosidase, luciferase, chloramphenicol

acetyltransferase, etc. that provides for convenient quantitation; and the like. The activity of the encoded ESE-1, ESE-2 or ESE-3 protein may be determined by comparison with the wild-type protein.

The invention relates to genotyping assays for SNPs in the ESE-1, ESE-2 or ESE-3 gene and immunoassays (e.g., ELISAs) specific for ESE-1, ESE-2 or ESE-3 as screening tools for diabetes (Type I and Type II), MS and other autoimmune disease predisposition. A variety of approaches for genotyping are known in the art. Such approaches include Taqman, PCR-based methods, Third Wave technology, Orchid Bioscience, Nanogen microelectronic chips, mass spectrometry-based methods (e.g., Sequenom, Qiagen) and the like.

The invention relates to genotyping assays for SNPs in the ESE-3 gene and other genes that are associated in complex traits in diabetes and other autoimmune diseases, including, but not limited to the ESE-1, ESE-2, lymphotoxin, TNF-α, CTLA-4, IL-10, TGF-β, Fc.epsilon receptor, Class I and Class II HLA antigens, T cell receptor and immunoglobulin genes, cytokines and cytokine receptors, chemokines and chemokine receptors and the like.

The invention relates to methods of interfering with the function or expression of ESE-1, ESE-2 or ESE-3 in patients who have a SNP in the ESE-1, ESE-2 or ESE-3 gene, respectively. Methods for interfering with the function or expression of ESE-1, ESE-2 or ESE-3 in patients include using small molecule drugs, peptides, gene delivery of dominant negative mutants, antisense and the like to block or enhance the function or expression of ESE-1, ESE-2 or ESE-3, respectively, systemically or locally, to prevent the development of diabetes (Type I or Type II), MS or other autoimmune diseases.

The invention relates to methods of blocking the function or expression of ESE-1, ESE-2 or ESE-3 during inflammation, which can result in a highly specific blockade of genes activated by ESE-1, ESE-2 or ESE-3, respectively, during inflammation. Inflammation includes inflammation associated with an inflammatory disease and other related diseases, and localized inflammation, such as that observed in restenosis after a coronary artery intervention procedure (e.g., balloon angioplasty) for the treatment of coronary artery disease or in the joints of

rheumatoid arthritis patients. Inflammatory diseases include rheumatoid arthritis, vascular inflammatory pathologies, such as, but not limited to, atherosclerosis, and chronic and acute inflammatory pathologies. Methods for blocking the function or expression of ESE-1, ESE-2 or ESE-3 include using small molecule drugs, peptides, gene delivery of dominant negative mutants, antisense and the like to block or enhance the function or expression of ESE-1, ESE-2 or ESE-3, respectively, systemically, to treat or prevent an inflammatory disease or other related disease, such as atherosclerosis or rheumatoid arthritis. Methods for blocking the function or expression of ESE-1, ESE-2 or ESE-3 further include local delivery of an ESE-1, ESE-2 or ESE-3 blocking or enhancing agent to treat localized inflammation, such as that observed in restenosis after a coronary artery intervention procedure (e.g., balloon angioplasty) for the treatment of coronary artery disease or in the joints of rheumatoid arthritis patients.

The invention relates to products for screening for diabetes, particularly Type I diabetes, and other autoimmune disease predisposition. Such products include kits, such as genotyping kits and immunoassay kits, including ELISA kits, that use ESE-1, ESE-2 or ESE-3 SNPs alone or in conjunction with SNPs in other genes due to complex trait associations.

The invention enables the use of ESE-1, ESE-2 or ESE-3 interfering agents to specifically target patients with a predisposing SNP in the ESE-1, ESE-2 or ESE-3 gene, respectively, and to treat inflammation in various inflammatory disease states, such as MS, rheumatoid arthritis and atherosclerosis, and in acute and chronic situations.

DIAGNOSTIC AND SCREENING ASSAYS

In one embodiment, diagnostic assays for assessing ESE-1, ESE-2 or ESE-3 gene expression, or for assessing activity of ESE-1, ESE-2 or ESE-3 polypeptides are used in the context of a biological sample (e.g., blood, serum, cells, tissue) to determine whether an individual is afflicted with diabetes or other autoimmune disease, or is at risk for (has a predisposition for or a susceptibility to) developing diabetes or other autoimmune disease. The invention also provides for prognostic

(or predictive) assays for determining whether an individual is susceptible to developing diabetes or other autoimmune disease. For example, mutations in the gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of symptoms associated with diabetes or other autoimmune disease. Another aspect of the invention pertains to assays for monitoring the influence of agents (e.g., drugs, compounds or other agents) on the gene expression or activity of polypeptides of the invention, as well as to assays for identifying agents which bind to ESE-1, ESE-2 or ESE-3 polypeptides. These and other assays and agents are described in further detail in the following sections.

DIAGNOSTIC ASSAYS

In one embodiment, diagnosis of a susceptibility to diabetes or other autoimmune disease is made by detecting a polymorphism in *ESE-1*. In a second embodiment, diagnosis of a susceptibility to diabetes or other autoimmune disease is made by detecting a polymorphism in *ESE-2*. In a third embodiment, diagnosis of a susceptibility to diabetes or other autoimmune disease is made by detecting a polymorphism in *ESE-3*. The polymorphism can be a mutation in *ESE-1*, *ESE-2* or *ESE-3*, such as the insertion or deletion of a single nucleotide, or of more than one nucleotide, resulting in a frame shift mutation; the change of at least one nucleotide, resulting in a change in the encoded amino acid; the change of at least one nucleotide, resulting in the generation of a premature stop codon; the change in at least one nucleotide in 3' or 5' untranslated regions, resulting in changes in RNA stability or translation; the change of at least one nucleotide in regulatory regions of the gene, resulting in changes in transcription or splicing; the deletion of several nucleotides, resulting in a deletion of one or more amino acids encoded by the nucleotides; the insertion of one or several nucleotides, such as by unequal recombination or gene conversion, resulting in an interruption of the coding sequence of the gene; duplication of all or a part of the gene; transposition of all or a part of the gene; or rearrangement of all or a part of the gene. More than one such mutation may be present in a single gene. Such sequence changes cause a mutation

in the polypeptide encoded by *ESE-1*, *ESE-2* or *ESE-3*. For example, if the mutation is a frame shift mutation, the frame shift can result in a change in the encoded amino acids, and/or can result in the generation of a premature stop codon, causing generation of a truncated polypeptide. Alternatively, a polymorphism associated with a susceptibility to autoimmune disease can be a synonymous mutation in one or more nucleotides (i.e., a mutation that does not result in a change in the polypeptide encoded by *ESE-1*, *ESE-2* or *ESE-3*). Such a polymorphism may alter splicing sites, affect the stability or transport of mRNA, or otherwise affect the transcription or translation of the gene. *ESE-1*, *ESE-2* or *ESE-3* that has any of the mutations described above is referred to herein as a "mutant gene." In a particular embodiment, the polymorphism is located in a regulatory region, such as a promoter region, of *ESE-1*, *ESE-2* or *ESE-3*.

In a particular embodiment, the polymorphism is a SNP at position -140 in the ESE-3 gene, and which is a guanine (G) nucleotide in comparison to an adenine (A) nucleotide in the wildtype ESE-3 gene. In another embodiment, the polymorphism is a SNP at position -4458 in the ESE-3 gene, and which is a thymine (T) nucleotide in comparison to a cytosine (C) nucleotide in the wildtype ESE-3 gene. In a third embodiment, the polymorphism is a SNP at position +171 in the ESE-1 gene, and which is a cytosine (C) nucleotide in comparison to a thymine (T) nucleotide in the wildtype ESE-1 gene. In a fourth embodiment, the polymorphism is a SNP at position +949 in the ESE-1 gene, and which is a thymine (T) nucleotide in comparison to a guanine (G) nucleotide in the wildtype ESE-1 gene. In a fifth embodiment, the polymorphism is a SNP at position +1275 in the ESE-1 gene, and which is an adenine (A) nucleotide in comparison to a cytosine (C) nucleotide in the wildtype ESE-1 gene. In a sixth embodiment, the polymorphism is a SNP at position +1639 in the ESE-1 gene, and which is an adenine (A) nucleotide in comparison to a guanine (G) nucleotide in the wildtype ESE-1 gene. In a seventh embodiment, the polymorphism is a SNP at position +1744 in the ESE-1 gene, and which is an adenine (A) nucleotide in comparison to a guanine (G) nucleotide in the wildtype ESE-1 gene. In an eighth embodiment, the polymorphism is a SNP at position -2034 in the ESE-1 gene, amd which is a cytosine (C) nucleotide in

comparison to a adenine (A) nucleotide in the wildtype ESE-1 gene. In a ninth embodiment, the polymorphism is a SNP at position -151 in the ESE-2 gene, and which is a cytosine (C) nucleotide in comparison to a guanine (G) nucleotide in the wildtype ESE-2 gene. Other sequence polymorphisms in the ESE-1, ESE-2 and ESE-3 genes are provided in Figure 1B. Additional embodiments of the invention relate to these sequence polymorphisms (SNPs).

In one method of diagnosing a susceptibility to diabetes, MS or other autoimmune disease, hybridization methods, such as Southern analysis, Northern analysis, or *in situ* hybridizations, can be used (see Current Protocols in Molecular Biology, Ausubel, F. *et al.*, eds., John Wiley & Sons, including all supplements through 1999). For example, a biological sample from a test subject (a "test sample") of genomic DNA, RNA, or cDNA, is obtained from an individual suspected of having, being susceptible to or predisposed for, or carrying a defect for, diabetes or other autoimmune disease (the "test individual"). The individual can be an adult, child, or fetus. The test sample can be from any source which contains genomic DNA, such as a blood sample, sample of amniotic fluid, sample of cerebrospinal fluid, or tissue sample from skin, muscle, buccal or conjunctival mucosa, placenta, gastrointestinal tract or other organs. A test sample of DNA from fetal cells or tissue can be obtained by appropriate methods, such as by amniocentesis or chorionic villus sampling. The DNA, RNA, or cDNA sample is then examined to determine whether a polymorphism in *ESE-1*, *ESE-2* or *ESE-3* is present, and/or to determine which variant(s) encoded by *ESE-1*, *ESE-2* or *ESE-3* are present. The presence of the polymorphism or variant(s) can be indicated by hybridization of the gene in the genomic DNA, RNA, or cDNA to an appropriate nucleic acid probe. An "appropriate nucleic acid probe", as used herein, can be a DNA probe or an RNA probe; the nucleic acid probe can contain at least one polymorphism in *ESE-1*, *ESE-2* or *ESE-3* or contains a nucleic acid encoding a particular variant of ESE-1, ESE-2 or ESE-3. The probe can be any nucleic acid molecule, such as the gene, a fragment or a vector comprising the gene.

To diagnose a susceptibility to diabetes, MS or other autoimmune disease, a hybridization sample is formed by contacting the test sample containing *ESE-1*,

ESE-2 or *ESE-3*, with at least one appropriate nucleic acid probe. A preferred probe for detecting mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to mRNA or genomic DNA sequences described herein. The nucleic acid probe can be, for example, a full-length nucleic acid molecule, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to appropriate mRNA or genomic DNA.

The hybridization sample is maintained under conditions which are sufficient to allow specific hybridization of the nucleic acid probe to *ESE-1*, *ESE-2* or *ESE-3*. "Specific hybridization", as used herein, indicates exact hybridization (e.g., with no mismatches). Specific hybridization can be performed under high stringency conditions or moderate stringency conditions, for example, as described above. In a particular embodiment, the hybridization conditions for specific hybridization are high stringency.

Specific hybridization, if present, is then detected using standard methods. If specific hybridization occurs between the relevant nucleic acid probe and *ESE-1*, *ESE-2* or *ESE-3* in the test sample, then *ESE-1*, *ESE-2* or *ESE-3*, respectively, has the polymorphism, or is the variant, that is present in the nucleic acid probe. More than one nucleic acid probe can also be used concurrently in this method. Specific hybridization of any one of the nucleic acid probes is indicative of a polymorphism in *ESE-1*, *ESE-2* or *ESE-3*, or of the presence of a particular variant encoded by *ESE-1*, *ESE-2* or *ESE-3*, and is therefore diagnostic for a susceptibility to diabetes or other autoimmune disease.

In Northern analysis (see Current Protocols in Molecular Biology, Ausubel, F. *et al.*, eds., John Wiley & Sons, *supra*), the hybridization methods described above are used to identify the presence of a polymorphism or of a particular variant, associated with a susceptibility to diabetes or other autoimmune disease. For Northern analysis, a test sample of RNA is obtained from the individual by appropriate means. Specific hybridization of an appropriate nucleic acid probe, as described above, to RNA from the individual is indicative of a polymorphism in *ESE-1*, *ESE-2* or *ESE-3*, or of the presence of a particular variant encoded by *ESE-1*,

ESE-2 or *ESE-3*, and is therefore diagnostic for a susceptibility to diabetes or other autoimmune disease.

For representative examples of use of nucleic acid probes, see, for example, U.S. Patents No. 5,288,611 and 4,851,330.

Alternatively, a peptide nucleic acid (PNA) probe can be used instead of a nucleic acid probe in the hybridization methods described above. PNA is a DNA mimic having a peptide-like, inorganic backbone, such as N-(2-aminoethyl)glycine units, with an organic base (A, G, C, T or U) attached to the glycine nitrogen via a methylene carbonyl linker (see, for example, Nielsen, P.E. *et al., Bioconjugate Chemistry*, 1994, 5, American Chemical Society, p. 1 (1994). The PNA probe can be designed to specifically hybridize to a gene having a polymorphism associated with a susceptibility to diabetes or other autoimmune disease. Hybridization of the relevant PNA probe to *ESE-1*, *ESE-2* or *ESE-3* is diagnostic for a susceptibility to autoimmune disease.

In another method of the invention, mutation analysis by restriction digestion can be used to detect a mutant gene, or genes containing a polymorphism(s), if the mutation or polymorphism in the gene results in the creation or elimination of a restriction site. A test sample containing genomic DNA is obtained from the individual. Polymerase chain reaction (PCR) can be used to amplify *ESE-1*, *ESE-2* or *ESE-3* (and, if necessary, the flanking sequences) in the test sample of genomic DNA from the test individual. RFLP analysis is conducted as described (see Current Protocols in Molecular Biology, *supra*). The digestion pattern of the relevant DNA fragment indicates the presence or absence of the mutation or polymorphism in *ESE-1*, *ESE-2* or *ESE-3*, and therefore indicates the presence or absence of this susceptibility to diabetes or other autoimmune disease.

Sequence analysis can also be used to detect specific polymorphisms in the ESE-1, ESE-2 or ESE-3 genes. A test sample of DNA or RNA is obtained from the test individual. PCR or other appropriate methods can be used to amplify the gene, and/or its flanking sequences, if desired. The sequence of *ESE-1*, *ESE-2* or *ESE-3*, or a fragment of the ESE-1, ESE-2 or ESE-3 gene, or an ESE-1, ESE-2 or ESE-3 cDNA, or fragment of the ESE-1, ESE-2 or ESE-3 cDNA, or an ESE-1, ESE-2 or

ESE-3 mRNA, or fragment of the ESE-1, ESE-2 or ESE-3 mRNA, is determined, using standard methods. The sequence of the gene, gene fragment, cDNA, cDNA fragment, mRNA, or mRNA fragment is compared with the known nucleic acid sequence of the ESE-1, ESE-2 or ESE-3 gene, cDNA or mRNA, as appropriate. The presence of a polymorphism in *ESE-1*, *ESE-2* or *ESE-3* indicates that the individual has a susceptibility to Type I diabetes, Type II diabetes, MS or other autoimmune disease.

Allele-specific oligonucleotides can also be used to detect the presence of a polymorphism in *ESE-1*, *ESE-2* or *ESE-3*, through the use of dot-blot hybridization of amplified oligonucleotides with allele-specific oligonucleotide (ASO) probes (see, for example, Saiki, R. *et al.*, (1986), *Nature (London) 324*:163-166). An "allele-specific oligonucleotide" (also referred to herein as an "allele-specific oligonucleotide probe") is an oligonucleotide of approximately 10-50 base pairs, preferably approximately 15-30 base pairs, that specifically hybridizes to *ESE-1*, *ESE-2* or *ESE-3*, and that contains a polymorphism associated with a susceptibility to diabetes or other autoimmune disease. An allele-specific oligonucleotide probe that is specific for particular polymorphisms in *ESE-1*, *ESE-2* or *ESE-3* can be prepared, using standard methods (see Current Protocols in Molecular Biology, *supra*). To identify polymorphisms in the gene that are associated with a susceptibility to diabetes or other autoimmune disease, a test sample of DNA is obtained from the individual. PCR can be used to amplify all or a fragment of *ESE-1*, *ESE-2* or *ESE-3*, and its flanking sequences. The DNA containing the amplified *ESE-1*, *ESE-2* or *ESE-3* (or fragment of the ESE-1, ESE-2 or ESE-3 cDNA gene) is dot-blotted, using standard methods (see Current Protocols in Molecular Biology, *supra*), and the blot is contacted with the oligonucleotide probe. The presence of specific hybridization of the probe to the amplified *ESE-1*, *ESE-2* or *ESE-3* is then detected. Specific hybridization of an appropriate allele-specific oligonucleotide probe to DNA from the individual is indicative of a polymorphism in *ESE-1*, *ESE-2* or *ESE-3*, and is therefore indicative of a susceptibility to diabetes or other autoimmune disease.

In another embodiment, arrays of oligonucleotide probes that are complementary to target nucleic acid sequence segments from an individual, can be used to identify polymorphisms in the ESE-1, ESE-2 or ESE-3 gene. For example, in one embodiment, an oligonucleotide array can be used. Oligonucleotide arrays typically comprise a plurality of different oligonucleotide probes that are coupled to a surface of a substrate in different known locations. These oligonucleotide arrays, also described as "Genechips.TM.," have been generally described in the art, for example, U.S. Pat. No. 5,143,854 and PCT patent publication Nos. WO 90/15070 and 92/10092. These arrays can generally be produced using mechanical synthesis methods or light directed synthesis methods which incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis methods. See Fodor et al., Science, 251:767-777 (1991), Pirrung et al., U.S. Pat. No. 5,143,854 (see also PCT Application No. WO 90/15070) and Fodor et al., PCT Publication No. WO 92/10092 and U.S. Pat. No. 5,424,186, the entire teachings of each of which are incorporated by reference herein. Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, e.g., U.S. Pat. Nos. 5,384,261, the entire teachings of which are incorporated by reference herein.

Once an oligonucleotide array is prepared, a nucleic acid of interest is hybridized with the array and scanned for polymorphisms. Hybridization and scanning are generally carried out by methods described herein and also in, e.g., Published PCT Application Nos. WO 92/10092 and WO 95/11995, and U.S. Pat. No. 5,424,186, the entire teachings of which are incorporated by reference herein. In brief, a target nucleic acid sequence which includes one or more previously identified polymorphic markers is amplified by well known amplification techniques, e.g., PCR. Typically, this involves the use of primer sequences that are complementary to the two strands of the target sequence both upstream and downstream from the polymorphism. Asymmetric PCR techniques may also be used. Amplified target, generally incorporating a label, is then hybridized with the array under appropriate conditions. Upon completion of hybridization and washing of the array, the array is scanned to determine the position on the array to which the

target sequence hybridizes. The hybridization data obtained from the scan is typically in the form of fluorescence intensities as a function of location on the array.

Although primarily described in terms of a single detection block, e.g., for detection of a single polymorphism, arrays can include multiple detection blocks, and thus be capable of analyzing multiple, specific polymorphisms. In alternate arrangements, it will generally be understood that detection blocks may be grouped within a single array or in multiple, separate arrays so that varying, optimal conditions may be used during the hybridization of the target to the array. For example, it may often be desirable to provide for the detection of those polymorphisms that fall within G-C rich stretches of a genomic sequence, separately from those falling in A-T rich segments. This allows for the separate optimization of hybridization conditions for each situation.

Additional description of use of oligonucleotide arrays for detection of polymorphisms can be found, for example, in U.S. Patents 5,858,659 and 5,837,832, the entire teachings of which are incorporated by reference herein.

Other methods of nucleic acid analysis can be used to detect polymorphisms in *ESE-1*, *ESE-2* or *ESE-3* or variants encoded by *ESE-1*, *ESE-2* or *ESE-3*. Representative methods include direct manual sequencing (Church and Gilbert, (1988), *Proc. Natl. Acad. Sci. USA 81*:1991-1995; Sanger, F. *et al.* (1977) *Proc. Natl. Acad. Sci. 74*:5463-5467; Beavis *et al.* U.S. Pat. No. 5,288,644); automated fluorescent sequencing; single-stranded conformation polymorphism assays (SSCP); clamped denaturing gel electrophoresis (CDGE); denaturing gradient gel electrophoresis (DGGE) (Sheffield, V.C. *et al.* (19891), *Proc. Natl. Acad. Sci. USA 86*:232-236), mobility shift analysis (Orita, M. *et al.* (1989), *Proc. Natl. Acad. Sci. USA 86*:2766-2770), restriction enzyme analysis (Flavell *et al.* (1978) *Cell 15*:25; Geever, *et al.* (1981), *Proc. Natl. Acad. Sci. USA 78*:5081); heteroduplex analysis; chemical mismatch cleavage (CMC) (Cotton *et al.* (1985), *Proc. Natl. Acad. Sci. USA 85*:4397-4401); RNase protection assays (Myers, R.M. *et al.* (1985), *Science 230*:1242); use of polypeptides which recognize nucleotide mismatches, such as *E. coli* mutS protein; allele-specific PCR, for example.

In another embodiment of the invention, diagnosis of a susceptibility to diabetes or other autoimmune disease can also be made by examining expression and/or composition of an ESE-1, ESE-2 or ESE-3 polypeptide, by a variety of methods, including enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. A test sample from an individual is assessed for the presence of an alteration in the expression and/or an alteration in composition of the polypeptide encoded by *ESE-1*, *ESE-2* or *ESE-3*, or for the presence of a particular variant encoded by *ESE-1*, *ESE-2* or *ESE-3*. An alteration in expression of a polypeptide encoded by the ESE-1, ESE-2 or ESE-3 gene can be, for example, an alteration in the quantitative polypeptide expression (i.e., the amount of polypeptide produced); an alteration in the composition of a polypeptide encoded by *ESE-1*, *ESE-2* or *ESE-3* is an alteration in the qualitative polypeptide expression (e.g., expression of a mutant ESE-1, ESE-2 or ESE-3 polypeptide or of a different variant). In a preferred embodiment, diagnosis of a susceptibility to diabetes or other autoimmune disease is made by detecting a particular variant encoded by *ESE-1*, *ESE-2* or *ESE-3*, or a particular pattern of variants.

Both quantitative and qualitative alterations can also be present. An "alteration" in the polypeptide expression or composition, as used herein, refers to an alteration in expression or composition in a test sample, as compared with the expression or composition of polypeptide encoded by *ESE-1*, *ESE-2* or *ESE-3* in a control sample. A control sample is a sample that corresponds to the test sample (e.g., is from the same type of cells), and is from an individual who is not affected by diabetes or other autoimmune disease. An alteration in the expression or composition of the polypeptide in the test sample, as compared with the control sample, is indicative of a susceptibility to diabetes or other autoimmune disease. Similarly, the presence of one or more different variants in the test sample, or the presence of significantly different amounts of different variants in the test sample, as compared with the control sample, is indicative of a susceptibility to diabetes or other autoimmune disease. Various means of examining expression or composition of the polypeptide encoded by the ESE-1, ESE-2 or ESE-3 genes can be used, including spectroscopy, colorimetry, electrophoresis, isoelectric focusing, and

immunoassays (e.g., David *et al.*, U.S. Pat. No. 4,376,110) such as immunoblotting (see also Current Protocols in Molecular Biology, particularly chapter 10). For example, in one embodiment, an antibody capable of binding to the polypeptide, preferably an antibody with a detectable label, can be used. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

Western blotting analysis, using an antibody that specifically binds to a polypeptide encoded by a mutant *ESE-1*, *ESE-2* or *ESE-3*, or an antibody that specifically binds to a polypeptide encoded by a non-mutant gene, or an antibody that specifically binds to a particular variant encoded by *ESE-1*, *ESE-2* or *ESE-3*, can be used to identify the presence in a test sample of a particular variant or of a polypeptide encoded by a polymorphic or mutant *ESE-1*, *ESE-2* or *ESE-3*, or the absence in a test sample of a particular variant or of a polypeptide encoded by a non-polymorphic or non-mutant gene. The presence of a polypeptide encoded by a polymorphic or mutant gene, or the absence of a polypeptide encoded by a non-polymorphic or non-mutant gene, is diagnostic for a susceptibility to diabetes or autoimmune disease, as is the presence (or absence) of particular variants encoded by the ESE-1, ESE-2 or ESE-3 gene.

In one embodiment of this method, the level or amount of polypeptide encoded by the ESE-1, ESE-2 or ESE-3 gene in a test sample is compared with the level or amount of the polypeptide encoded by the ESE-1, ESE-2 or ESE-3 gene, respectively, in a control sample. A level or amount of the polypeptide in the test sample that is higher or lower than the level or amount of the polypeptide in the control sample, such that the difference is statistically significant, is indicative of an

alteration in the expression of the polypeptide encoded by *ESE-1*, *ESE-2* or *ESE-3*, respectively, and is diagnostic for a susceptibility to diabetes or other autoimmune disease. Alternatively, the composition of the polypeptide encoded by *ESE-1*, *ESE-2* or *ESE-3* in a test sample is compared with the composition of the polypeptide encoded by *ESE-1*, *ESE-2* or *ESE-3*, respectively, in a control sample. A difference in the composition of the polypeptide in the test sample, as compared with the composition of the polypeptide in the control sample (e.g., the presence of different variants), is diagnostic for a susceptibility to diabetes or other autoimmune disease. In another embodiment, both the level or amount and the composition of the polypeptide can be assessed in the test sample and in the control sample. A difference in the amount or level of the polypeptide in the test sample, compared to the control sample; a difference in composition in the test sample, compared to the control sample; or both a difference in the amount or level, and a difference in the composition, is indicative of a susceptibility to diabetes or other autoimmune disease.

Kits (e.g., reagent kits) useful in the methods of diagnosis comprise components useful in any of the methods described herein, including for example, hybridization probes or primers as described herein (e.g., labeled probes or primers), reagents for detection of labeled molecules, restriction enzymes (e.g., for RFLP analysis), allele-specific oligonucleotides, antibodies which bind to mutant or to non-mutant (native) ESE-1, ESE-2 or ESE-3, means for amplification of nucleic acids comprising *ESE-1*, *ESE-2* or *ESE-3*, or means for analyzing the nucleic acid sequence of *ESE-1*, *ESE-2* or *ESE-3* or for analyzing the amino acid sequence of an ESE-1, ESE-2 or ESE-3 polypeptide, etc.

SCREENING ASSAYS AND AGENTS IDENTIFIED THEREBY

In another embodiment, the invention provides methods for identifying agents (e.g., fusion proteins, polypeptides, peptidomimetics, prodrugs, receptors, binding agents, antibodies, small molecules or other drugs, or ribozymes) which alter (e.g., increase, decrease, enhance or block) the activity or function of the polypeptides described herein, or which otherwise interact with the polypeptides

herein. For example, such agents can be agents which bind to polypeptides described herein (e.g., ESE-1, ESE-2 or ESE-3 binding agents); which have a stimulatory or inhibitory effect on, for example, activity or function of ESE-1, ESE-2 or ESE-3 polypeptides; which change (e.g., enhance or inhibit or block) the ability of the ESE-1, ESE-2 or ESE-3 polypeptide to interact with ESE-1, ESE-2 or ESE-3 binding agents (e.g., receptors or other binding agents); or which alter posttranslational processing of the ESE-1, ESE-2 or ESE-3 polypeptide (e.g., agents that alter proteolytic processing to direct the polypeptide from where it is normally synthesized to another location in the cell, such as the cell surface; agents that alter proteolytic processing such that more active polypeptide is released from the cell, etc.).

In one embodiment, the invention provides assays for screening candidate or test agents that bind to or modulate the activity or function of an ESE-1. ESE-2 or ESE-3 polypeptide (or biologically active portion(s) thereof), as well as agents identifiable by the assays. Test agents can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S., (1997), *Anticancer Drug Des., 12*:145).

In one embodiment, to identify agents which alter the activity or function of an ESE-1, ESE-2 or ESE-3 polypeptide, a cell, cell lysate, or solution containing or expressing an ESE-1, ESE-2 or ESE-3 polypeptide, or a fragment or derivative thereof, can be contacted with an agent to be tested; alternatively, the polypeptide can be contacted directly with the agent to be tested. The level (amount) of ESE-1, ESE-2 or ESE-3 activity is assessed (e.g., the level (amount) of ESE-1, ESE-2 or ESE-3 activity is measured, either directly or indirectly), and is compared with the level of activity in a control (i.e., the level of activity of the ESE-1, ESE-2 or ESE-3

polypeptide or fragment or derivative thereof in the absence of the agent to be tested). If the level of the activity in the presence of the agent differs, by an amount that is statistically significant, from the level of the activity in the absence of the agent, then the agent is an agent that alters the activity of ESE-1, ESE-2 or ESE-3 polypeptide. An increase in the level of ESE-1, ESE-2 or ESE-3 activity relative to a control, indicates that the agent is an agent that enhances (is an agonist of) ESE-1, ESE-2 or ESE-3 activity. Similarly, a decrease in the level of ESE-1, ESE-2 or ESE-3 activity relative to a control, indicates that the agent is an agent that inhibits (is an antagonist of) ESE-1, ESE-2 or ESE-3 activity. In another embodiment, the level of activity of an ESE-1, ESE-2 or ESE-3 polypeptide or derivative or fragment thereof in the presence of the agent to be tested, is compared with a control level that has previously been established. A level of the activity in the presence of the agent that differs from the control level by an amount that is statistically significant indicates that the agent alters ESE-1, ESE-2 or ESE-3 activity.

The present invention also relates to an assay for identifying agents which alter the expression of the ESE-1, ESE-2 or ESE-3 gene (e.g., antisense nucleic acids, fusion proteins, polypeptides, peptidomimetics, prodrugs, receptors, binding agents, antibodies, small molecules or other drugs, or ribozymes), which alter (e.g., increase, decrease, enhance or block) expression (e.g., transcription or translation) of the gene or which otherwise interact with the nucleic acids described herein, as well as agents identifiable by the assays. For example, a solution containing a nucleic acid encoding ESE-1, ESE-2 or ESE-3 polypeptide (e.g., ESE-1, ESE-2 or ESE-3 gene) can be contacted with an agent to be tested. The solution can comprise, for example, cells containing the nucleic acid or cell lysate containing the nucleic acid; alternatively, the solution can be another solution which comprises elements necessary for transcription/translation of the nucleic acid. Cells not suspended in solution can also be employed, if desired. The level and/or pattern of *ESE-1*, *ESE-2* or *ESE-3* expression (e.g., the level and/or pattern of mRNA or of protein expressed, such as the level and/or pattern of different variants) is assessed, and is compared with the level and/or pattern of expression in a control (i.e., the level and/or pattern of the *ESE-1*, *ESE-2* or *ESE-3* expression in the absence of the agent to be tested). If

the level and/or pattern in the presence of the agent differs, by an amount or in a manner that is statistically significant, from the level and/or pattern in the absence of the agent, then the agent is an agent that alters the expression of *ESE-1*, *ESE-2* or *ESE-3*. Enhancement of ESE-1, ESE-2 or ESE-3 gene expression indicates that the agent is an agonist of ESE-1, ESE-2 or ESE-3 gene activity. Similarly, inhibition of *ESE-1*, *ESE-2* or *ESE-3* expression indicates that the agent is an antagonist of *ESE-1*, *ESE-2* or *ESE-3* activity. In another embodiment, the level and/or pattern of ESE-1, ESE-2 or ESE-3 polypeptide(s) (e.g., different variants) in the presence of the agent to be tested, is compared with a control level and/or pattern that has previously been established. A level and/or pattern in the presence of the agent that differs from the control level and/or pattern by an amount or in a manner that is statistically significant indicates that the agent alters ESE-1, ESE-2 or ESE-3 expression.

In another embodiment of the invention, agents which alter the expression of the ESE-1, ESE-2 or ESE-3 gene or which otherwise interact with the nucleic acids described herein, can be identified using a cell, cell lysate, or solution containing a nucleic acid encoding the promoter region of the ESE-1, ESE-2 or ESE-3 gene operably linked to a reporter gene. After contact with an agent to be tested, the level of expression of the reporter gene (e.g., the level of mRNA or of protein expressed) is assessed, and is compared with the level of expression in a control (i.e., the level of the expression of the reporter gene in the absence of the agent to be tested). If the level in the presence of the agent differs, by an amount or in a manner that is statistically significant, from the level in the absence of the agent, then the agent is an agent that alters the expression of *ESE-1*, *ESE-2* or *ESE-3*, as indicated by its ability to alter expression of a gene that is operably linked to the ESE-1, ESE-2 or ESE-3 gene promoter. Enhancement of the expression of the reporter indicates that the agent is an agonist of *ESE-1*, *ESE-2* or *ESE-3* activity. Similarly, inhibition of the expression of the reporter indicates that the agent is an antagonist of *ESE-1*, *ESE-2* or *ESE-3* activity. In another embodiment, the level of expression of the reporter in the presence of the agent to be tested, is compared with a control level that has previously been established. A level in the presence of the agent that differs

from the control level by an amount or in a manner that is statistically significant indicates that the agent alters *ESE-1*, *ESE-2* or *ESE-3* expression.

Agents which alter the amounts of different variants encoded by *ESE-1*, *ESE-2* or *ESE-3* (e.g., an agent which enhances activity of a first variant, and which inhibits activity of a second variant), as well as agents which are agonists of activity of a first variant and antagonists of activity of a second variant, can easily be identified using these methods described above.

In other embodiments of the invention, assays can be used to assess the impact of a test agent on the activity or function of an ESE-1, ESE-2 or ESE-3 polypeptide in relation to an ESE-1, ESE-2 or ESE-3 binding agent. For example, a cell that expresses a compound that interacts with ESE-1, ESE-2 or ESE-3 (herein referred to as a "ESE-1, ESE-2 or ESE-3 binding agent" or "ESE-1, ESE-2 or ESE-3 interfering agent", which can be a polypeptide or other molecule that interacts with ESE-1, ESE-2 or ESE-3, such as a receptor) is contacted with ESE-1, ESE-2 or ESE-3 in the presence of a test agent, and the ability of the test agent to alter the interaction between ESE-1, ESE-2 or ESE-3 and the ESE-1, ESE-2 or ESE-3 binding agent, respectively, is determined. Alternatively, a cell lysate or a solution containing the ESE-1, ESE-2 or ESE-3 binding agent, can be used. An agent which binds to ESE-1, ESE-2 or ESE-3 or the ESE-1, ESE-2 or ESE-3 binding agent can alter the interaction by interfering with, or enhancing the ability of ESE-1, ESE-2 or ESE-3 to bind to, associate with, or otherwise interact with the ESE-1, ESE-2 or ESE-3 binding agent, respectively. Determining the ability of the test agent to bind to ESE-1, ESE-2 or ESE-3 or an ESE-1, ESE-2 or ESE-3 binding agent can be accomplished, for example, by coupling the test agent with a radioisotope or enzymatic label such that binding of the test agent to the polypeptide can be determined by detecting the labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test agents can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. It is also within the scope of this invention to determine the ability of a

test agent to interact with the polypeptide without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a test agent with ESE-1, ESE-2 or ESE-3 or an ESE-1, ESE-2 or ESE-3 binding agent without the labeling of either the test agent, ESE-1, ESE-2 or ESE-3, or the ESE-1, ESE-2 or ESE-3 binding agent. McConnell, H.M. *et al.*, (1992), *Science, 257*:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between ligand and polypeptide.

In another embodiment of the invention, assays can be used to identify polypeptides that interact with one or more ESE-1, ESE-2 or ESE-3 polypeptides, as described herein. For example, a yeast two-hybrid system such as that described by Fields and Song (Fields, S. and Song, O., *Nature, 340*:245-246 (1989)) can be used to identify polypeptides that interact with one or more ESE-1, ESE-2 or ESE-3 polypeptides. In such a yeast two-hybrid system, vectors are constructed based on the flexibility of a transcription factor which has two functional domains (a DNA binding domain and a transcription activation domain). If the two domains are separated but fused to two different proteins that interact with one another, transcriptional activation can be achieved, and transcription of specific markers (e.g., nutritional markers such as His and Ade, or color markers such as lacZ) can be used to identify the presence of interaction and transcriptional activation. For example, in the methods of the invention, a first vector is used which includes a nucleic acid encoding a DNA binding domain and also an ESE-1, ESE-2 or ESE-3 polypeptide, variant, or fragment or derivative thereof, and a second vector is used which includes a nucleic acid encoding a transcription activation domain and also a nucleic acid encoding a polypeptide which potentially may interact with the ESE-1, ESE-2 or ESE-3 polypeptide, variant, or fragment or derivative thereof (e.g., a ESE-1, ESE-2 or ESE-3 polypeptide binding agent or receptor). Incubation of yeast containing the first vector and the second vector under appropriate conditions (e.g., mating conditions such as used in the Matchmaker™ system from Clontech) allows

identification of colonies which express the markers of interest. These colonies can be examined to identify the polypeptide(s) which interact with the ESE-1, ESE-2 or ESE-3 polypeptide or fragment or derivative thereof. Such polypeptides may be useful as agents which alter the activity or expression of an ESE-1, ESE-2 or ESE-3 polypeptide, as described above.

In more than one embodiment of the above assay methods, it may be desirable to immobilize either ESE-1, ESE-2 or ESE-3, the ESE-1, ESE-2 or ESE-3 binding agent, or other components of the assay on a solid support, in order to facilitate separation of complexed from uncomplexed forms of one or both of the polypeptides, as well as to accommodate automation of the assay. Binding of a test agent to the polypeptide, or interaction of the polypeptide with a binding agent in the presence and absence of a test agent, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein (e.g., a glutathione-S-transferase fusion protein) can be provided which adds a domain that allows ESE-1, ESE-2 or ESE-3 or an ESE-1, ESE-2 or ESE-3 binding agent to be bound to a matrix or other solid support.

In another embodiment, modulators of expression of nucleic acid molecules of the invention are identified in a method wherein a cell, cell lysate, or solution containing a nucleic acid encoding ESE-1, ESE-2 or ESE-3 is contacted with a test agent and the expression of appropriate mRNA or polypeptide (e.g., variant(s)) in the cell, cell lysate, or solution, is determined. The level of expression of appropriate mRNA or polypeptide(s) in the presence of the test agent is compared to the level of expression of mRNA or polypeptide(s) in the absence of the test agent. The test agent can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater (statistically significantly greater) in the presence of the test agent than in its absence, the test agent is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less (statistically significantly less) in the presence of the test agent than in its absence, the test agent is identified as an inhibitor of the mRNA or

polypeptide expression. The level of mRNA or polypeptide expression in the cells can be determined by methods described herein for detecting mRNA or polypeptide.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a test agent that is a modulating agent, an antisense nucleic acid molecule, a specific antibody, or a polypeptide-binding agent) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above described screening assays for treatments as described herein.

METHODS OF THERAPY

The present invention also pertains to methods of treatment (prophylactic and/or therapeutic) for diabetes or other autoimmune disease in individuals having a polmorphism in the ESE-1, ESE-2 or ESE-3 gene, using an ESE-1, ESE-2 or ESE-3 therapeutic agent, respectively. An "ESE-1, ESE-2 or ESE-3 therapeutic agent" is an agent that alters (e.g., enhances, inhibits or blocks) ESE-1, ESE-2 or ESE-3 polypeptide activity or function and/or ESE-1, ESE-2 or ESE-3 gene expression, as described herein (e.g., an ESE-1, ESE-2 or ESE-3 agonist or antagonist). ESE-1, ESE-2 or ESE-3 therapeutic agents can alter ESE-1, ESE-2 or ESE-3 polypeptide activity or function or gene expression by a variety of means, such as, for example, by providing additional ESE-1, ESE-2 or ESE-3 polypeptide or by upregulating the transcription or translation of the ESE-1, ESE-2 or ESE-3 gene; by altering post-translational processing of the ESE-1, ESE-2 or ESE-3 polypeptide; by altering transcription of ESE-1, ESE-2 or ESE-3 variants; or by interfering with ESE-1, ESE-2 or ESE-3 polypeptide activity or function (e.g., by binding to an ESE-1, ESE-2 or ESE-3 polypeptide); or by enhancing ESE-1, ESE-2 or ESE-3 polypeptide activity or function; or by downregulating the transcription or translation of the ESE-1, ESE-2

or ESE-3 gene. Representative ESE-1, ESE-2 or ESE-3 therapeutic agents include the following:

nucleic acids or fragments or derivatives thereof described herein, particularly nucleotides encoding the polypeptides described herein and vectors comprising such nucleic acids (e.g., a gene, cDNA, and/or mRNA, such as a nucleic acid encoding an ESE-1, ESE-2 or ESE-3 polypeptide or active fragment or derivative thereof); antisense; double stranded iRNA;

polypeptides described herein;

other polypeptides (e.g., ESE-1, ESE-2 or ESE-3 receptors); ESE-1, ESE-2 or ESE-3 binding agents; peptidomimetics; fusion proteins or prodrugs thereof; antibodies (e.g., an antibody to a mutant ESE-1, ESE-2 or ESE-3 polypeptide, or an antibody to a non-mutant ESE-1, ESE-2 or ESE-3 polypeptide, or an antibody to a particular variant encoded by *ESE-1*, *ESE-2* or *ESE-3*); ribozymes; small molecule drugs; other small molecules;

dominant negative mutants;

and other agents that alter (e.g., enhance or inhibit) ESE-1, ESE-2 or ESE-3 gene expression or polypeptide activity or function, that alter post-translational processing of the ESE-1, ESE-2 or ESE-3 polypeptide, or that regulate transcription of ESE-1, ESE-2 or ESE-3 variants (e.g., agents that affect which variants are expressed, or that affect the amount of each variant that is expressed).

In a particular embodiment, the ESE-1, ESE-2 or ESE-3 therapeutic agent is a nucleic acid encoding one or more ESE-1, ESE-2 or ESE-3 polypeptides. In another embodiment, the ESE-1, ESE-2 or ESE-3 therapeutic agent is a nucleic acid comprising a fragment of the ESE-1, ESE-2 or ESE-3 gene, such as a regulatory region of the ESE-1, ESE-2 or ESE-3 gene. In yet another embodiment, the ESE-1, ESE-2 or ESE-3 therapeutic agent is a nucleic acid comprising the ESE-1, ESE-2 or ESE-3 gene regulatory region and also a nucleic acid encoding one or more ESE-1, ESE-2 or ESE-3 polypeptides (or fragments or derivatives thereof). In another embodiment, the ESE-1, ESE-2 or ESE-3 therapeutic agent is a small molecule drug. In yet another embodiment, the ESE-1, ESE-2 or ESE-3 therapeutic agent is a dominant negative mutant, antisense molecule or peptide.

More than one ESE-1, ESE-2 or ESE-3 therapeutic agent can be used concurrently, if desired.

The term "treatment", as used herein, refers not only to ameliorating symptoms associated with the disease or disorder, but also preventing or delaying the onset of the disease or disorder, and also lessening the severity or frequency of symptoms of the disease. The therapy is designed to alter (e.g., inhibit or enhance), replace or supplement activity of an ESE-1, ESE-2 or ESE-3 polypeptide in an individual. For example, an ESE-1, ESE-2 or ESE-3 therapeutic agent can be administered in order to upregulate or increase the expression or availability of the ESE-1, ESE-2 or ESE-3 gene or of specific variants of ESE-1, ESE-2 or ESE-3, or, conversely, to downregulate or decrease the expression or availability of the ESE-1, ESE-2 or ESE-3 gene or specific variants of ESE-1, ESE-2 or ESE-3. Upregulation or increasing expression or availability of a native ESE-1, ESE-2 or ESE-3 gene or of a particular variant could interfere with or compensate for the expression or activity or function of a defective gene or another variant; downregulation or decreasing expression or availability of a native ESE-1, ESE-2 or ESE-3 gene or of a particular variant could minimize the expression or activity or function of a defective gene or the particular variant and thereby minimize the impact of the defective gene or the particular variant.

The ESE-1, ESE-2 or ESE-3 therapeutic agent(s) are administered in a therapeutically effective amount (i.e., an amount that is sufficient to treat the disease or disorder, such as by ameliorating symptoms, such as inflammation, associated with the disease or disorder, preventing or delaying the onset of the disease or disorder, and/or also lessening the severity or frequency of symptoms of the disease or disorder). The amount which will be therapeutically effective in the treatment of a particular individual's disorder or condition will depend on the symptoms and severity of the disease, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of a practitioner and each patient's

circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

In one embodiment of the invention, a nucleic acid of the invention; a nucleic acid complementary to a nucleic acid of the invention; or a portion of such a nucleic acid (e.g., an oligonucleotide as described below), can be used in "antisense" therapy, in which a nucleic acid (e.g., an oligonucleotide) which specifically hybridizes to the mRNA and/or genomic DNA of *ESE-1*, *ESE-2* or *ESE-3* is administered or generated *in situ*. The antisense nucleic acid that specifically hybridizes to the mRNA and/or DNA inhibits expression of the ESE-1, ESE-2 or ESE-3 polypeptide, e.g., by inhibiting translation and/or transcription. Binding of the antisense nucleic acid can be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interaction in the major groove of the double helix.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid. When the plasmid is transcribed in the cell, it produces RNA which is complementary to a portion of the mRNA and/or DNA which encodes ESE-1, ESE-2 or ESE-3 polypeptide. Alternatively, the antisense construct can be an oligonucleotide probe which is generated *ex vivo* and introduced into cells; it then inhibits expression by hybridizing with the mRNA and/or genomic DNA of *ESE-1*, *ESE-2* or *ESE-3*. In one embodiment, the oligonucleotide probes are modified oligonucleotides which are resistant to endogenous nucleases, e.g. exonucleases and/or endonucleases, thereby rendering them stable *in vivo*. Exemplary nucleic acid molecules for use as antisense oligonucleotides are phosphoramidate, phosphothioate and methylphosphonate analogs of DNA (see also U.S. Pat. Nos. 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy are also described, for example, by Van der Krol *et al.* ((1988) *Biotechniques* 6:958-976); and Stein *et al.* ((1988) *Cancer Res* 48:2659-2668).

To perform antisense therapy, oligonucleotides (mRNA, cDNA or DNA) are designed that are complementary to mRNA encoding ESE-1, ESE-2 or ESE-3. The antisense oligonucleotides bind to ESE-1, ESE-2 or ESE-3 mRNA transcripts,

respectively, and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, indicates that a sequence has sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid, as described in detail above. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures.

The oligonucleotides used in antisense therapy can be DNA, RNA, or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotides can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotides can include other appended groups such as peptides (e.g. for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.*, (1989), *Proc. Natl. Acad. Sci. USA*, 86:6553-6556; Lemaitre *et al.*, (1987), *Proc. Natl. Acad Sci. USA*, 84:648-652; PCT International Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT International Publication No. W089/10134), or hybridization-triggered cleavage agents (see, e.g., Krol *et al.*, (1988), *BioTechniques*, 6:958-976) or intercalating agents. (See, e.g., Zon, (1988), *Pharm. Res.*, 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent).

The antisense molecules are delivered to cells which express ESE-1, ESE-2 or ESE-3 having a polymorphism therein *in vivo*. A number of methods can be used for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that

specifically bind receptors or antigens expressed on the target cell surface) can be administered systematically. Alternatively, in a preferred embodiment, a recombinant DNA construct is utilized in which the antisense oligonucleotide is placed under the control of a strong promoter (e.g., pol III or pol II). The use of such a construct to transfect target cells in the patient results in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous *ESE-1*, *ESE-2* or *ESE-3* transcripts and thereby prevent translation of the *ESE-1*, *ESE-2* or *ESE-3* mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art and described above. For example, a plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site. Alternatively, viral vectors can be used which selectively infect the desired tissue, in which case administration may be accomplished by another route (e.g., systematically).

Endogenous *ESE-1*, *ESE-2* or *ESE-3* expression can also be reduced by inactivating or "knocking out" *ESE-1*, *ESE-2* or *ESE-3* or its promoter using targeted homologous recombination (e.g., see Smithies *et al.*, (1985), *Nature*, 317:230-234; Thomas & Capecchi, (1987), *Cell*, 51:503-512; Thompson *et al.*, (1989), *Cell*, 5:313-321). For example, a mutant, non-functional ESE-1, ESE-2 or ESE-3 gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous ESE-1, ESE-2 or ESE-3 gene (either the coding regions or regulatory regions of *ESE-1*, *ESE-2* or *ESE-3*) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells *in vivo* that express *ESE-1*, *ESE-2* or *ESE-3* having a polymorphism therein. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the ESE-1, ESE-2 or ESE-3 gene. The recombinant DNA constructs can be directly administered or targeted to the required site *in vivo* using appropriate vectors, as described above. Alternatively, expression of non-mutant *ESE-1*, *ESE-2* or *ESE-3* can be increased

using a similar method: targeted homologous recombination can be used to insert a DNA construct comprising a non-mutant, functional *ESE-1*, *ESE-2* or *ESE-3*, or a portion thereof, in place of a mutant *ESE-1*, *ESE-2* or *ESE-3* in the cell, as described above. In another embodiment, targeted homologous recombination can be used to insert a DNA construct comprising a nucleic acid that encodes an ESE-1, ESE-2 or ESE-3 polypeptide variant that differs from that present in the cell.

Alternatively, endogenous ESE-1, ESE-2 or ESE-3 gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of *ESE-1*, *ESE-2* or *ESE-3* (i.e., the *ESE-1*, *ESE-2* or *ESE-3* promoter and/or enhancers) to form triple helical structures that prevent transcription of *ESE-1*, *ESE-2* or *ESE-3* in target cells in the body. (See generally, Helene, C., (1991), *Anticancer Drug Des.*, 6(6):569-84; Helene, C., *et al.*, (1992), *Ann, N.Y. Acad. Sci.*, 660:27-36; and Maher, L. J., (1992), *Bioassays*, 14(12):807-15). Likewise, the antisense constructs described herein, by antagonizing the normal biological activity of one of the ESE-1, ESE-2 or ESE-3 proteins, can be used in the manipulation of tissue, e.g. tissue differentiation, both *in vivo* and *for ex vivo* tissue cultures. Furthermore, the antisense techniques (e.g. microinjection of antisense molecules, or transfection with plasmids whose transcripts are antisense with regard to an *ESE-1*, *ESE-2* or *ESE-3* mRNA or gene sequence) can be used to investigate role of ESE-1, ESE-2 or ESE-3 in developmental events, as well as the normal cellular function of ESE-1, ESE-2 or ESE-3 in adult tissue. Such techniques can be utilized in cell culture, but can also be used in the creation of transgenic animals.

In yet another embodiment of the invention, other ESE-1, ESE-2 or ESE-3 therapeutic agents as described herein can also be used in the treatment or prevention of diabetes or autoimmune disease. The therapeutic agents can be delivered in a composition or by themselves. They can be administered systemically, or can be targeted to a particular tissue. The therapeutic agents can be produced by a variety of means, including chemical synthesis; recombinant production; *in vivo* production (e.g., a transgenic animal, such as U.S. Pat. No. 4,873,316 to Meade *et al.*), for example, and can be isolated using standard means such as those described herein.

A combination of any of the above methods of treatment (e.g., administration of non-mutant ESE-1, ESE-2 or ESE-3 polypeptide in conjunction with antisense therapy targeting mutant *ESE-1*, *ESE-2* or *ESE-3* mRNA; administration of a first variant encoded by *ESE-1*, *ESE-2* or *ESE-3* in conjunction with antisense therapy targeting a second variant encoded by *ESE-1*, *ESE-2* or *ESE-3*), can also be used.

The invention will be further described by the following non-limiting examples. The teachings of all publications cited herein are incorporated herein by reference in their entirety.

EXAMPLES

EXAMPLE 1 Correlation of Several SNPs with Diabetes.

The distribution of SNPs in the genes of TNF-α, Lymphotoxin (LT), CTLA4, TGFβ IL-6P and the ets factor ESE-3 was investigated. Two separated populations were studied. First, a collection of DNA from Bahrain, divided in three groups: diabetic type I, diabetic type II and control subjects. The second study population consisted of asthma patients and control subjects from Boston.

The ESE-3 gene polymorphism A/G in position -140 is found in 27% of the healthy and type 2 diabetic population but in 44% of the type 1 diabetic population (Figures 3A-3D, 4A-4B, 5A-5B, 6A-6E and 7-8). The ESE-3 gene polymorphism C/T in position -4458 is found in 26% of the healthy population, 31% of the type 1 population and 32% of the type 2 population (Figures 6A-6E and 7). The TNF-α SNP G/A in position -308 is found in between 11 and 19% of the healthy and type 2 diabetic population but in 26% of the type 1 population. Similarly, the SNP on 249 A/G in the LT gene is present in between 48 and 54% in healthy and type 2 diabetic population but in 63% of the type 1.

Significant differences in the ESE-3 SNP (-140) proportion are found between different populations. Forty-four percent of the Boston population present this SNP for only 27% of the Bahrain population.

The results from a statistical X^2 analysis are shown in Figures 4A-4B, 5A-5B and 8. The probability (p value) of association of a SNP in the genes as indicated

with either type I or type II diabetes was calculated. The closer the p value is to 0 the higher the probability. The results demonstrate that the highest probability of linkage was for ESE-3 (-140) with Type I diabetes with a p value of 0.082, followed by lymphotoxin LT 249 with a p value of 0.16. No significant association of ESE-3 gene polymorphism A/G in position -140 was found with Type II diabetes.

EXAMPLE 2 Correlation of SNPs with Multiple Sclerosis (MS).

The study population consisted of 87 MS patients and 30 control subjects (Figures 9A-9C and 10). The ESE-3 gene polymorphism A/G at position -140 is found in 24% of the healthy population and 41% of the MS population (Figures 9A-9C and 10). The results indicate a linkage for ESE-3 (-140) with MS.

The following materials and methods were used in the work described in Example 3.

Cell Culture and Patient Samples.

U87 Mg and U138 (human glioma), THP-1 (human monocytic), RAW 264.7 (murine monocytic), LB-12 (human osteoblast-like large T antigen transformed bone marrow stromal cells) and immortalized human chondrocytes were grown and treated with cytokines as described previously. IL-1β, TNF-α, and IFN-γ were purchased from R&D Systems.

Tissues from patients with rheumatoid arthritis (RA) were obtained as discarded materials from total joint replacement surgery or synovectomy. Tissue procurement was approved by the Institutional Review Board. RA synovial tissue samples were either immediately snap frozen for RNA extraction, or cultured for the generation of adherent synovial fibroblasts. Dispersed synovial tissues were prepared by a previously published method. Briefly, synovial tissues were minced on tissue culture plates and treated with Type I collagenase (4 mg/ml; Worthington Biochemical Corporation, Lakewood, NJ), in Dulbecco Modification of Eagle medium (Gibco BRL), incubated for 1 hour at 37°C, treated with 0.25% trypsin for 30 minutes, harvested and centrifuged at 1,000 rpm for 10 minutes. Pellets were

suspended in 0.05% trypsin-0.02% EDTA for 10 minutes, centrifuged and resuspended in 50% Phosphate Buffered Saline, 50% DMEM containing 10% fetal calf serum (Sigma, St Louis, MO). Cells were then centrifuged and suspended in DMEM, 10% FCS and plated at a density of 10 x 10^6 cells/10 cm plate. Cells were initially grown for 7-10 days and subsequently subjected to 2-4 passages. Passaged cells were stimulated with IL-1β (R&D Systems) at 100 pg/ml or with TNF-α (R&D Systems) at 5 ng/ml.

Expression Vector and Luciferase Reporter Gene Constructs.

PCR was used to generate the ESE-3 promoter reporter constructs. Construct 1463ESE3 includes nucleotides from bp –1463 up to bp +94 of exon 1, and was generated using the sense primer 5'-TGACCAAAGCCTCAAGCCCTTC-3' (SEQ ID NO:57) and the antisense primer 5'-AAAGATTCTGGGTGGGAAATCCAA-3' (SEQ ID NO:58). Construct 1019ESE3 contains nucleotides from bp –1019 up to +94 bp of exon 1, and was generated using the sense primer 5'-CATTTGTGACCCAGTGAAGCAGG-3' (SEQ ID NO:59) and the same antisense primer as the former construct. Both PCR products were shuttled via the TA cloning vector pCRII (Invitrogen), sequence verified and cloned in the blunted XhoI site of the basal luciferase vector pXP2. Construct 694ESE3 was generated by digesting 1463ESE3 construct with KpnI and religation. Construct 444ESE3 was generated by digesting 1019ESE3 with SmaI and ApaI and religation.

Electrophoretic Mobility Shift Assays (EMSA).

EMSAs and supershift assays were performed as described (Lopez, M. et al., Mol. Cell. Biol., 14:3292-3309 (1994)) using 2 μl in vitro translation product or 3 μl of whole cell extract and 0.1-0.2 ng [^{32}P]-labeled double stranded oligonucleotide probes (5000-20000 cpm) in the presence or absence of competitor oligonucleotides (1 ng and 10 ng) or antibodies and run on 4% polyacrylamide gels, containing as buffer 0.5 x TGE.

In vitro transcription-translation was performed using the TNT rabbit reticulocyte lysate kit (Promega) and the pCI-ESE-3 vector as described (Kas, K. et

al., J. Biol. Chem., 275:2986-2998 (2000)). The lysis buffer for whole cell extract contains: 1% Triton X-100, 25 mM glycylglycine pH7.8, 15 mM $MgSO_4$, 4 mM EGTA, 1 mM dithiothreitol, phenylmethylsulfonyl fluoride, Aprotinin and Pepstatin. All antibodies for supershift assays (2 µl per assay) were obtained from Santa Cruz Biotechnology (CA) (Gu, X. et al., J. Biol. Chem., 276:9421-9436 (2001)). Oligonucleotides for competition and/or direct binding studies are as follows:

ESE3P1
NF-κB site WT
5'-GTGACTTAGGGAATTCCCGGTTGGTG-3' (SEQ ID NO:60)
5'-CACCAACCGGGAATTCCCTAAGTCAC-3' (SEQ ID NO:61)

ESE3P1
NF-κB site MUT
5'-GGGAAGGTGACTTACCGAATTCCCGGTTGG-3' (SEQ ID NO:62)
5'-CCAACCGGGAATTCGGTAAGTCACTTCCC-3' (SEQ ID NO:63)

Site-directed Mutagenesis.

Mutations in the ESE-3 promoter NF-κB site were generated by site-directed mutagenesis with the QuikChange Site-directed Mutagenesis kit (Stratagene) and confirmed by sequencing. Sequences of the wild type ESE-3/NF-κB site and the mutation introduced within the ESE-3 promoter:

ESE3P1
NF-κB site WT
5'-GTGACTTAGGGAATTCCCGGTTGGTG-3' (SEQ ID NO:64)
5'-CACCAACCGGGAATTCCCTAAGTCAC-3' (SEQ ID NO:65)

ESE3P1

NF-κB site MUT

5'-GGGAAGGTGACTTACCGAATTCCCGGTTGG-3' (SEQ ID NO:66)

5'-CCAACCGGGAATTCGGTAAGTCACTTCCC-3' (SEQ ID NO:67)

DNA Transfection Assays.

Cotransfections of 3-8x10^5 Raw or T/C24I2 were carried out with 2 μg reporter gene construct DNA and 3 μg expression vector DNA using 12.5 μl lipofectamine (Gibco-BRL) as described (Oettgen, P. et al., Genomics, 55(3):358-362 (1999)). The cells were harvested 16 hours after transfection and assayed for luciferase activity (Libermann, T.A. et al., Mol. Cell. Biol., 10(5):2327-2334 (1990)). Transfections for every construct were performed independently in duplicates and repeated 3 times with two different plasmid preparations with similar results. Cotransfection of a second plasmid for determination of transfection efficiency was omitted because potential artifacts with this technique have been reported (Bondeson, J. et al., J. Rheumatol., 27(9):2078-2089 (2000)) and because many commonly used viral promoters contain potential binding sites for Ets factors.

RT-PCR Analysis.

Total RNA was harvested using QIAshreder (Qiagen) and RNeasy® Mini Kit (Quiagen) or Trizol (Gibco BRL). Poly A^+ RNA was prepared with MicroPoly(A) Pure (Ambion).

cDNAs were generated from 1 μg of total RNA using Ready-To-Go™ You-prime First-Strand Beads (Amersham Pharmacia Biotech Inc). RT-PCR amplifications of 0.1 μg cDNA were carried out using a MJResearch thermal cycler PTC-100 as follows: 5 minutes at 94°C, 20-37 cycles of 30 seconds at 94°C, 30 seconds at 56°C and 30 seconds at 72°C, followed by 5 minutes at 72°C (Oettgen, P. et al., J. Biol. Chem., 275(2):1216-1225 (2000)). The sequences of the human ESE-3 primers and the primers for human GAPDH were as previously described (Oettgen, P. et al., J. Biol. Chem., 274(41):29439-29452 (1999)). The sequences of the human ESE-3 gene are as follows:

5'-ttc aaa tga gat tgt ggg aaa att gct-3' (sense bases 574-600) (SEQ ID NO:68)

5'-aga tca tct ctg cct gag tat ctt-3' (antisense bases 855-878) (SEQ ID NO:69)

Adenovirus Infection.

T/C28a2 chondrocyte cells were infected with the IκB adenovirus (kindly provided by Fionula Brennan) and adenovirus expressing null vector, or β-galactosidase adenovirus (Bondeson, J. et al., Proc. Natl. Acad. Sci. USA, 96(10):5668-5673 (1999)) for 1 hour in serum-free medium using a multiplicity of infection of 1000. After infection the cells were washed with medium and incubated for 4 hours in DMEM containing 10% fetal calf serum in the absence or presence of IL-1β.

Real-Time Quantitative PCR.

SYBR Green I-based real-time PCR was carried out on an Opticon Monitor (MJ Research, Inc, Waltham, MA). All PCR mixtures contained PCR buffer [final concentration: 10 mM Tris-HCl (pH9.0), 50 mM KCl, 2mM $MgCl_2$ and 0.1% Triton X-100], 250 uM deoxy-NTP (Roche), 0.5 uM of each PCR primer, 0.5X SYBR Green I, 5% DMSO and 1U Taq DNA polymerase (Promega, Madison, WI) with 2 ul cDNA in a 25 ul final volume of reaction mix. The samples were loaded into wells of Low Profile 96-well microplates. After an initial denaturation step at 95°C for 2 minutes, conditions for cycling were 38 cycles of denaturation (95°C for 30 seconds), annealing (54°C for 30 seconds) and extension (72°C for 1 minutes). Fluorescence signal was measured immediately following incubation at 78°C for 5 seconds that follows each extension step, which eliminates possible primer dimer detection. At the end of PCR cycles, a melting curve was generated to identify specificity of PCR product. For each run, serial dilutions of human GAPDH plasmids were used as standards for quantitative measurement of the amount of amplified cDNA. For normalization of each sample, hGAPDH primers were used to measure the amount of hGAPDH cDNA. All samples were run as duplicates and the data were presented as ratio of ESE-3/hGAPDH and then as a fold increase to the first control sample. The primers used for real time PCR are as follows: for

hGAPDH, forward: 5'-CAA AGT TGT CAT GGA TGA CC (SEQ ID NO:70), reverse: 5'-CCA TGG AGA AGG CTG GGG (SEQ ID NO:71), which amplify 195 bp of human GAPDH. ESE3 primers were the same as described above for RT-PCR analysis and amplify 408 bp of human ESE-3.

Western Blot Analysis.

$4x10^5$ T/C 28I2 chondrocyte cells were plated per well 16 hours before being exposed to IL-1β in fresh medium for different periods of time. The cells were rinsed with PBS and harvested in 200 ul of lysis buffer containing protease inhibitors (Roche), then frozen and thawed once prior to sonication. The nuclear protein was extracted. 40 ul of lysate were loaded onto a 10% SDS polyacrylamide gel. Proteins were transferred to a PVDF membrane, blocked with 5% milk in PBS/Tween (0.2%), and incubated with a polyclonal antibody directed against fulllength ESE-3 followed by HRP-conjugated secondary antibodies. Immunoreactive bands were detected using ECL reagent (Pharmacia) following the manufacturer's instructions.

Chromatin Immunoprecipitation (ChIP).

THP-1 human monocytic cells were grown in RPMI 1640 medium containing 10% FBS (low LPS, from Hyclone), 0.05mM β-mercaptoethanol and 1% penicillin/streptomycin (BRL-Life Technologies) in a 5% CO_2 incubator. Cells ($8x 10^6$) were plated on 100mm dishes and then stimulated with LPS (1μg/ml) for 1 hour. Cross-linking was performed by adding formaldehyde directly to tissue culture medium to a final concentration of 1% and incubating for 10 minutes at room temperature. Cells then were rinsed twice with ice-cold PBS, and collected into 100 mM Tris-HCl (pH 9.4), 10 mM DTT, incubated for 15 minutes at 30°C and centrifuged for 5 minutes at 2000 X g. Cells were washed sequentially with 1 ml of ice-cold PBS, buffer I (0.25% Triton X-100, 10 mM EDTA, 0.5 mM EGTA, 10 mM HEPES, pH 6.5), and buffer II (200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 10 mM HEPES, pH 6.5). Cells were resuspended in 0.3 ml of lysis buffer (1% SDS, 5 mM EDTA, 50 mM Tris-HCl, pH 8.1, 1X protease inhibitor cocktail (Roche

Molecular Biochemicals, Indianapolis, IN)), incubated on ice for 10 minutes and then sonicated three times for 30 seconds each at 1 minute intervals at the maximum setting to make 400bp – 1 kb DNA fragmentation, followed by centrifugation for 10 minutes at 14,000 Xg at 4°C. Supernatants were collected and 100 µl of chromatin preparation was aliquoted as the input fraction. The remaining supernatant was diluted in buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, 1X protease inhibitor cocktail pH 7.9) followed by immunoclearing with 2 µg sheared salmon sperm DNA, 20 µl normal rabbit serum and protein A-Sepharose (45 µl of 50% slurry in 10 mM Tris-HCl, pH8.1, 1 mM EDTA, Amersham Pharmacia Biotech) for 2 hour at 4°C. Immunoprecipitation was performed overnight at 4°C with 0.5 µg of specific antibody, NF-κB p65 (Santa Cruz), or with 0.5 µg of rabbit IgG as a negative control. After immunoprecipitation, 45 µl protein A-Sepharose and 2 µg of sheared salmon sperm DNA were added and the incubation was continued for another hour. Precipitates were washed sequentially for 10 minutes each in TSE I (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 150 mM NaCl), TSE II (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 500 mM NaCl), and TSE III (0.25 M LiCl, 1% NP-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH8.0). Precipitates were then washed three times with $T_{10}E_1$ buffer (pH 8.0) and extracted three times with 1% SDS, 0.1 M $NaHCO_3$. Eluates were pooled and heated at 65°C overnight to reverse the formaldehyde cross-linking. DNA fragments were purified with QIAquick PCR Purfication Kit (Qiagen, CA). PCR was performed using 2 µl of a 50 µl DNA extraction in TE buffer with Hi-Fi Taq polymerase (Invitrogen). PCR mixtures were amplified for 1 cycle of 94°C for 2 minutes, and 35-38 cycles of 95°C for 30 seconds, annealing temperature of 54°C for 30 seconds and 72°C for 1 minute, followed by a final elongation step at 72°C for 8 minutes. The sequences of the primers used are as follows: hESE3/P-F1, 5'-CAA ATG CAA ATG AGC CAA TG (SEQ ID NO:72) and hESE3/P-R1, 5'-GGG CGT TAT CAA GTC TGA GC (SEQ ID NO:73) which amplify 196 bp of the hESE3 promoter and TLR4/P-F1, 5'-CAT TGC ACT TGC TAC TTT CCA (SEQ ID NO:74) and TLR4/P-R1,

5'-CGC ATG TGT TTT GAA TTA CTG AA (SEQ ID NO:75) which amplify 215 bp of the hTLR4 promoter.

EXAMPLE 3 The Ets Factor ESE-3, A Novel Potential Transcriptional Mediator of Inflammation.

ESE-1, ESE-2 and ESE-3, three members of the ESE (Epithelial Specific Ets transcription factors) branch of the ETS transcription factor family, are, under normal physiological conditions, only expressed in epithelial tissues. However, it was recently shown that ESE-1 expression is rapidly and transiently induced by pro-inflammatory stimuli in both vascular and connective tissue cell types and at sites of inflammation.

As demonstrated by the experiments described herein, ESE-3 is inducible by IL-1β, TNF-α and LPS in various non-epithelial cells including synovial fibroblasts, chondrocytes, monocytes, as well as in glial cells. The upregulation of ESE-3 by pro-inflammatory cytokines and endotoxin suggests that this transcription factor is involved in transducing cell responses elicited by these proinflammatory mediators. ESE-3 induction requires activation of the NF-κB p50 and p65 family members. As described herein, NF-κB p50 and p65 bind with high affinity to the ESE-3 promoter NF-κB site upon stimulation by IL-1β. In addition, a mutation of the ESE-3 promoter NF-κB site abolishes the ability of IL-1 to transactivate the ESE-3 promoter. Moreover, the expression of a physiological inhibitor of NF-κB, IκB, prevents the induction of endogenous ESE-3 expression by IL-1. The results herein demonstrate that ESE-3 may play a role as a mediator of the inflammatory response in connective tissue cells.

Introduction

Inflammation is the body's natural response to help fight infection. However, in many circumstances the inflammatory response, if it continues too long or too severe, can do more harm than good. The cause of an inflammatory disease is either that the inflammatory process gets out of balance, or that the immune system turns on itself to attack the very tissues it was designed by evolution to protect. Seemingly

unrelated disorders such as asthma, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, chronic obstructive pulmonary disease, allergic rhinitis and cardiovascular disease, all include inflammatory elements (Marok, R. et al., Arthritis Rheum., 39:583-591 (1996); Miagkov, A. et al., Proc. Natl. Acad. Sci. USA, 95:13859-13864 (1998); and Neurath, M. et al., Nat. Med., 2:998-1004 (1996)).

Inflammatory processes contribute to the chronic or acute pathological events in autoimmune and other inflammatory conditions that lead to tissue injury and destruction. Rheumatoid arthritis (RA) is a prototypical immune-mediated disease characterized by chronic inflammation in the synovium and the destruction of joints in which, similar to other inflammatory disorders, a central role for interleukin (IL)-1 and tumor necrosis factor (TNF)-α has been established. These cytokines, similar to bacterial endotoxins, have major roles in inflammatory responses via the activation of a variety of transcription factors, including NF-κB, NFAT, AP-1 and C/EBP family members. One of the major transcriptional circuits implicated in inflammation is the NF-κB/IκB pathway. NF-κB is rapidly activated by proinflammatory cytokines such as IL-1 and TNF-α and endotoxins and is involved in the regulation of a large set of inflammatory response genes, including various cytokines and chemokines, acute phase proteins, cell adhesion proteins and immunoglobulins. Although many of these genes are directly regulated by NF-κB via high affinity binding sites within their respective promoter regions, this mechanism does not account exclusively for the regulation of a significant number of inflammatory response genes. Thus, additional pathways play critical roles in the transcriptional regulation of these genes.

The role of the Ets transcription factor family in epithelial cell differentiation is of particular interest. Three of them, ESE-1 (Oettgen, P. et al., Mol. Cell. Biol., 17:4419-4433 (1997)), ESE-2 (Oettgen, P. et al., J. Biol. Chem., 274:29439-29452 (1999)) and ESE-3 (Kas, K. et al., J. Biol. Chem., 275:2986-2998 (2000)), belong to a new subfamily originally defined by their high sequence homology and the restriction of their expression to epithelial cells. Recently, it has been demonstrated that ESE-1 expression can be induced by pro-inflammatory cytokines in other tissue

types as well, indicating that Ets factors may also play a role in inflammatory processes outside the epithelial cell lineage. ESE-1 is expressed in the synovium of rheumatoid arthritis patients and in the endothelial and vascular smooth muscle cells during endotoxemia (Rudders, S. et al., J. Biol. Chem., 276(5):3302-3309 (2001)), although it is not expressed significantly in these tissues under normal physiological conditions. Induction of ESE-1 by pro-inflammatory stimuli is largely dependent on activation by the NF-κB p50 and p65 family members, which induce ESE-1 expression via a high affinity NF-κB binding site within the ESE-1 promoter.

The NF-κB/Rel transcription factor family, including NF-κB 1 (p50/p105), NF-κB 2 (p52/p100), p65 (RelA), RelB and c-Rel (Chen, F. et al., Clin. Chem., 45:7-17 (1999)), plays a central role in numerous chronic inflammatory diseases and has been confirmed as a crucial and evolutionarily conserved factor in the inflammatory response (Tak, P.P. et al., J. Clin. Invest., 107:7-11 (2001)). Most members of this family (RelB being one exception) can form homodimers, as well as heterodimers with other members. The most prevalent form of NF-κB that is activated by pro-inflammatory stimuli such as LPS, IL-1β or TNF-α is a heterodimer consisting of a p50 or p52 subunit and p65 (Schottelius, A.J. et al., J. Biol. Chem., 274:31868-31874 (1999). NF-κB is present as an inactive, cytoplasmic factor bound to its inhibitor IκB and becomes activated by nuclear translocation of its subunits after degradation of IκB. The nuclear localization of NF-κB leads to the induction of Cox-2 and inducible nitric oxide synthase (iNOS), as well as various MMPs, pro-inflammatory cytokines, chemokines, acute phase proteins and cell adhesion molecules. Cytokines that activate NF-κB, such as IL-1β and TNF-α, can also be directly stimulated by the NF-κB pathway, thus establishing a positive autoregulatory loop that can amplify the inflammatory response and increase the duration of inflammation. Furthermore, NF-κB interacts and synergizes with various other transcription factors such as C/EBP, AP-1, HMG(I) and Sp-1 in regulating the expression of its targets genes.

A few Ets transcription factors including Ets-1, Ets-2 and Pu.1 have been reported to be involved in inflammatory processes. Several inflammation response genes contain putative Ets binding sites within their regulatory regions, and some of

them have been shown to depend on Ets transcription factors for their inducibility by cytokines such as IL-1β or TNF-α.

Connective tissue cell types including synovial fibroblasts, osteoblast-like bone marrow stromal cells, chondrocytes, monocytes/macrophages, as well as glial cells, are important targets of cytokines and endotoxin in inflammatory diseases such as RA. These cell types are used to evaluate the transcription factors involved in inflammatory and destructive processes. Having identified ESE-1 as a participant in the inflammatory process, the two other members of the ESE sub-family, ESE-2 and ESE-3, were evaluted to determine if they behave similarly.

As described herein, it has been discovered that expression of ESE-3 is transiently induced by pro-inflammatory cytokines in synovial fibroblasts, chondrocytes and monocytes. Both ESE-1 induction and ESE-3 induction by IL-1β, TNF-α and LPS are controlled via the NF-κB pathway. Stimulation of ESE-3 expression by these pro-inflammatory factors implicates ESE-3 as a potentially important regulatory molecule in inflammatory processes. Thus, ESE-3, similar to NF-κB, may play a role in mediating the effects of pro-inflammatory cytokines and endotoxin in non-epithelial cell types present at sites of inflammation.

Results

ESE-3 expression is induced by pro-inflammatory cytokines IL-1β, TNF-α and LPS in non-epithelial cells.

Although Northern blot hybridization and in situ hybridization previously demonstrated the restriction of ESE family expression to epithelial cells under normal physiological conditions, ESE-1, the founding member of the ESE family of Ets factors, was recently shown to be expressed in vascular and connective tissue cells in response to pro-inflammatory cytokines (Rudders, S. et al., J. Biol. Chem., 276(5):3302-3309 (2001)). The possibility that the other two members of the ESE family, ESE-2 and ESE-3, may also be targets for pro-inflammatory stimuli was examined.

Due to the apparent involvement of ESE-1 in rheumatoid arthritis, the behavior of ESE-2 and ESE-3 expression in connective tissue related cell types was

analyzed. Towards this end, fourth passage primary synovial fibroblasts isolated from the synovium of RA patients were cultured for different times in the absence or presence of the pro-inflammatory cytokine IL-1β. RT/PCR analysis of RNA isolated from these synovial fibroblasts revealed that unstimulated cells did not express significant levels of ESE-3 mRNA. In contrast, IL-1β stimulated cells demonstrated a pronounced transient ESE-3 transcript induction that remained detectable over several days (Figure 11A). ESE-1 expression was strongly upregulated by IL-1β within 6 hours. After 24 hours, expression declined and at 5 days was very low, although still above basal levels. ESE-2 expression was undetectable by RT-PCR under either condition. These results demonstrate that in addition to ESE-1, pro-inflammatory cytokines induced a transient expression of ESE-3, but not ESE-2, in synovial fibroblasts. Induction of ESE-3 by IL-1β was not restricted to synovial fibroblasts, since ESE-3 expression was also induced by IL-1β in three human chondrocyte cell lines, T/C28 a2, T/C28 i2 and C20A4 (Figure 11B).

To determine whether other pro-inflammatory cytokines affect ESE-3 expression, ESE-3 expression in response to a variety of cytokines in several connective tissue cell types was analyzed. Three immortalized human costal chondrocytes cell lines, T/C28I2, T/C28a2 and C20A4, were stimulated with IL-1β, TNF-α or IFN-γ for 24 hours. IL-1β and TNF-α induced ESE-3 expression in T/C28I2 and T/C28a2 cells, but not in C20A4 cells (Figure 11B). In contrast, IFN-γ was unable to induce ESE-3 expression in any of the three cell lines (Figure 11B), indicating that some, but not all, proinflammatory cytokines are capable of inducing ESE-3 expression.

The kinetics of ESE-3 induction was explored in a time course experiment (0.5, 2, 6, 12 and 24 hours) with the human costal chondrocyte cell line T/C28a2. RT/PCR analysis revealed rapid induction of ESE-3 mRNA by both IL-1β and TNF-α in a dose-dependent and time-dependent manner, reaching a peak within two to six hours and declining gradually thereafter (Figure 11C). The response to IL-1β was much stronger than to TNF-α. In contrast, RT/PCR analysis revealed that ESE-3 expression was not induced by IFN-γ.

Monocytes play a key role in the initiation of inflammation. In response to injurious agents such as bacterial endotoxin lipopolysaccharide (LPS), monocytes secrete "early response" cytokines that trigger the activation of other macrophages and resident connective tissue cells such as endothelial cells, chondrocytes, osteoblasts and synovial fibroblasts. To elucidate the involvement of ESE-3 in this process, the human monocytic cell line THP-1 was stimulated with bacterial endotoxin LPS for 0, 1, 4, 6 and 24 hours (Figure 11D). ESE-3 was prominently expressed within 6 hours after stimulation, indicating that endotoxins are additional potent inducers of ESE-3 and that monocytes are also able to express ESE-3 upon activation by pro-inflammatory stimuli.

The ESE-3 promoter is inducible by pro-inflammatory stimuli.

To investigate the molecular mechanism by which LPS and cytokines, such as IL-1β, regulates ESE-3 expression, the response of an ESE-3 promoter luciferase construct after transfection into THP-1 monocytes was examined. Briefly, the ESE-3 promoter was cloned into a vector containing the luciferase reporter gene. THP-1 monocytes were transiently transfected with the ESE-3 promoter luciferase reporter construct and incubated with LPS for 16 hours at 10 ng/ml. LPS stimulation resulted in a five-fold activation of the ESE-3 promoter (Figure 12A).

Since the molecules that can induce ESE-3 are known activators of NF-κB, to determine whether NF-κB was the intermediate between these activators and the ESE-3 promoter, expression vectors containing NF-κB family members p65 or p50 were cotransfected with an ESE-3 promoter luciferase reporter construct into Raw cells. A 3-fold induction of ESE-3 promoter activity was obtained upon co-transfection with p65 (Figure 12B).

Inducibility of the ESE-3 promoter by pro-inflammatory stimuli is mediated via a NF-κB site and the NF-κB/rel family members p50 and p65.

NF-κB has been shown to be a critical regulatory molecule involved in transducing cell responses to IL-1β and endotoxin. Sequencing analysis of the ESE-3 promoter revealed one potential NFκB binding site between the nucleotides

-119 and -110. Using EMSA, the ability of the ESE-3 NF-κB site and the ability of the ESE-1 promoter NF-κB site to form complexes with proteins present in whole cell extracts from unstimulated and IL-1β stimulated U-138 MG cells were compared. It was observed that the ESE-3 promoter NF-κB site probe was able to form a DNA-protein complex specifically with IL-1β treated U-138 MG extracts. This complex migrated at the same level as a complex formed by the ESE-1 promoter NF-κB site and was not present when extracts from unstimulated U-138 MG cells were used. The complex formed with the ESE-3 NF-κB site was significantly stronger than the one formed with the ESE-1 NF-κB site, suggesting that the ESE-3 promoter NF-κB site may be a very high affinity-binding site for NF-κB. A supershift assay using antibodies directed against the different NF-κB family members p65, p50, RelB, p52, c-rel demonstrated that this complex is constituted specifically of p50 and p65 (Figure 13). Increasing amounts of unlabeled ESE-3 NF-κB competitor oligonucleotide abolished the inducible complex, while a mutant ESE-3 NF-κB competitor oligonucleotide had no effect.

To confirm that the NF-κB site is responsible for IL-1β/LPS-mediated activation of the ESE-3 gene, a mutation replacing the 3' CCC with CGG was introduced into the ESE-3/NF-κB site (see Site-directed Mutagenesis above). The wild type and mutant ESE-3 promoter/luciferase plasmids were transfected into RAW monocytes and incubated in the absence or presence of LPS. This 3'mutation almost completely abolished the ability of the ESE-3 promoter to respond to LPS stimulation (Figure 14). Thus, an intact NF-κB binding site is essential for induction of ESE-3 by LPS and the inducibility of the ESE-3 gene by pro-inflammatory stimuli can be explained, at least in part, by activation by NF-κB.

NF-κB binds the endogenous ESE3 promoter.

To determine if NF-κB binds to ESE-3 promoter *in vivo*, a chromatin immunoprecipitation (ChIP) assay was performed in THP-1 human monocytic cells. Briefly, the cells were stimulated with 1 μg/ml of LPS for 0, 0.5, 1 or 3 hours and then treated with formaldehyde to crosslink the transcription factors to the promoter

DNA. Immunoprecipitation of the protein-DNA complexes with an antibody against NF-κB p65 was followed by analysis of the immunoprecipitated DNA by PCR using promoter-specific primers spanning the NF-κB site of ESE3. Fractionation of chromatin with anti-NF-κB p65 antibody, but not the control rabbit IgG, specifically enriched for endogenous ESE-3 promoter DNA. Furthermore, without LPS stimulation, NF-κB p65 had little or no binding to the endogenous ESE3 promoter. However, with LPS stimulation, endogenous NF-κB p65 binding to ESE3 promoter in THP-1 cells was evident after 0.5 hours. As a negative control, PCR using primers to amplify the DNA from the TLR4 locus which does not encompass any possible NF-κB binding sites showed no enriched bands with anti-NF-κB p65 antibody.

Inhibition of NF-κB activation by an adenovirus expressing IκB blocks IL-1β mediated induction of endogenous ESE-3 expression.

To further examine whether LPS and cytokines such as IL-1β mediate ESE-3 gene expression via NF-κB, NF-κB activation was blocked with overexpression of IκB, the physiologic inhibitor of NF-κB. Chondrocyte T/C20a2 cells were infected with either an adenovirus expressing IκB or an adenovirus expressing β-galactosidase as a control and subsequently stimulated with IL-1β for 6 hours. The IκB adenovirus drastically inhibited ESE-3 induction by IL-1β. These data confirm that NF-κB activation is a critical step involved in ESE-3 induction by IL-1β, although additional factors may contribute to ESE-3 induction.

ESE-3 protein levels were increased following the incubation with IL-1β.

Chondrocyte T/C20a2 cells were cultured in the absent or present of IL-1β for 0, 2, 4, 8, 12 or 24 hours. Total RNA and nuclear protein were extracted from those cells. Real-time quantitative RT-PCR for ESE-3 mRNA expression revealed that induction for ESE-3 specific mRNA expression reached its peak at 12 hours after IL-1 stimulation. Thereafter, ESE-3 expression steadily declined, although it was still partially present at 24 hours. Similarly, Western blot analysis of

chondrocyte T/C20a2 cells stimulated by IL-1β for different times revealed that IL-1β transiently induced ESE-3 expression after 1 hour incubation. ESE-3 expression peaked at 12 hours and declined after 18 hours. The expression level of ESE-3 protein correlated well with its mRNA levels.

Discussion

In numerous pathological conditions such as rheumatoid arthritis, multiple sclerosis, asthma, inflammatory bowel disease, *Helicobacter* pylori-associated gastritis and systemic inflammatory response syndrome, cytokines TNF-α and IL-1 are of prime importance in eliciting an inflammatory response (Tak, P.P. et al., J. Clin. Invest., 107: 7-11 (2001); and Romas, E. et al., Bone, 30:340-346 (2002)). Similarly, during infection, the bacterial endotoxin LPS is a major inducer of an inflammatory reaction that can result in dramatic situations such as septic shock. These compounds produce both their immediate and long term effects through activation of the NF-kB pathway (Loghmani, F. et al., Inflammation, 26:73-82 (2002); Kurosaka, K. et al., Cellular Immunology, 211:1-7 (2001); and Kikuchi, T. et al., J. Antimicrobial Chemotherapy, 49:745-755 (2002)). Animal models of inflammatory arthritis also support the notion that NF-κB activation plays a pathogenic role in vivo (Han, Z.N. et al., Autoimmunity, 28:197-208 (1998); Tsao, P.W. et al., Clin. Immunol. Immunopathol., 83:173-178 (1997); and Tak, P.P. et al., Arthritis Rheum., 42:S400 (Abstract) (1999)).

A novel Ets transcription factor, ESE-3 (also named EHF), whose expression under normal physiological conditions is restricted to epithelial cells was recently discovered. In surveying other cell types in which ESE-3 could play a potential regulatory role, an unexpected function for ESE-3 in non-epithelial cells including synovial fibroblasts, chondrocytes, monocyte/macrophages and glial cells was discovered. Attention was focused on these cell types because of their importance as targets of cytokines and endotoxin in inflammatory diseases such as RA and certain nervous system disorders. As described herein, ESE-3 expression was discovered to be induced transiently in response to inflammatory stimuli such as IL-1β, TNF-α and endotoxin in these non-epithelial cells. Thus, it is expected that ESE-3 functions as

a novel mediator of the inflammatory response. As described herein, induction of ESE-3 expression by pro-inflammatory stimuli was also found to be, in large part, dependent on activation of NF-κB, which induces ESE-3 expression via a high affinity NF-κB binding site within the ESE-3 promoter. These results firmly place ESE-3 as a downstream target for NF-κB.

The potent and transient ESE-3 induction by inflammatory cytokines appears to be distinct from most other members of the Ets family, since analysis of 18 additional Ets family members revealed only two additional Ets factors, ESE-1 and Ets-1, inducible by IL-1β in U-138 MG cells. ESE-3, therefore, represents a novel target for the treatment of inflammatory disorders such as rheumatoid arthritis.

Both ESE-3 and ESE-1 can be activated by IL-1, TNF-α and LPS. In contrast, IFN-γ fails to activate ESE-1 or ESE-3, indicating that not every pro-inflammatory compound can elicit this same response. Although ChIP assays and EMSAs indicate that NF-κB binds with much higher affinity to the ESE-3 promoter than it does to the promoter of ESE-1, both of them are downstream targets of NF-kB and depend on NF-kB to respond to pro-inflammatory signals.

Although both ESE-3 and ESE-1 can be rapidly and transiently induced in non-epithelial cells in response to inflammatory stimuli, a comparison of their expression kinetics reveals that ESE-1 is activated prior to ESE-3. For example, in IL-1 treated synovial fibroblasts, the ESE-3 expression peaks within a few days while ESE-1 reaches its highest expression level after only 6 hours. Selective activation of ESE-1 and ESE-3 seems to be involved in synovial inflammation. The temporal coordination of ESE-1 and ESE-3 expression in this manner may provide such cells with a mechanism for both short-term induction and long-term persistence of the inflammatory response.

The induction of ESE-3 and ESE-1 appears to occur through activation of NF-kB by translocation of this factor from the cytoplasm to the nucleus. This is supported by the observation that the ESE-3 promoter can bind in vivo to p65 and that mutation of this site abolishes the IL-1 mediated induction of ESE-3. The production of ESE-3, in turn, modulates other genes that may already be direct targets of NF-kB or additional players in the inflammatory process. Other Ets

factors directly interact and cooperate with NF-κB, so it is expected that ESE-3 cooperates with NF-κB to enhance the inflammatory response. ESE-3 target genes represent only a subset of inflammatory response genes and may define a functionally related class of genes. Thus, ESE-3 may be an alternative target for anti-inflammatory drugs, permitting blockage of more specific aspects of inflammation than would be possible by blocking activation of NF-kB.

The results described herein establish an important role for ESE-3 in inflammation. The role of ESE-3 as a pro-inflammatory factor can be characterized and its downstream targets can be identified to allow for development of novel and efficacious therapeutics for a wide variety of inflammatory diseases.

The teachings of all the articles, patents and patent applications cited herein are incorporated by reference in their entirety.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A method of diagnosis of autoimmune disease or of a predisposition to autoimmune disease or of diagnosing a susceptibility to autoimmune disease comprising detecting a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene, wherein detection of said polymorphism is indicative of the occurrence of autoimmune disease or a predisposition to autoimmune disease or a susceptibility to autoimmune disease.

2. A method of diagnosis of diabetes or of a predisposition to diabetes or of diagnosing a susceptibility to diabetes comprising detecting a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene, wherein detection of said polymorphism is indicative of the occurrence of diabetes or a predisposition to diabetes or a susceptibility to diabetes.

3. A method of diagnosis of multiple sclerosis or of a predisposition to multiple sclerosis or of diagnosing a susceptibility to multiple sclerosis comprising detecting a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene, wherein detection of said polymorphism is indicative of the occurrence of multiple sclerosis or a predisposition to multiple sclerosis or a susceptibility to multiple sclerosis.

4. The method of any one of Claim 1, 2 or 3 wherein said polymorphism is selected from the group consisting of: a SNP at position -140 and a SNP at position -4458.

5. The method of any one of Claim 1, 2 or 3 wherein said polymorphism is selected from the group consisting of:

a) a SNP at position −140 and said SNP is a guanine (G) nucleotide in comparison to an adenine (A) nucleotide in the wildtype ESE-3 gene; and

b) a SNP at position −4458 and said SNP is a thymine (T) nucleotide in comparison to a cytosine (C) nucleotide in the wildtype ESE-3 gene.

6. A method of diagnosis of autoimmune disease or of a predisposition to autoimmune disease or of diagnosing a susceptibility to autoimmune disease comprising detecting a polymorphism in the ESE-1 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-1 gene, wherein detection of said polymorphism is indicative of the occurrence of autoimmune disease or a predisposition to autoimmune disease or a susceptibility to autoimmune disease.

7. A method of diagnosis of diabetes or of a predisposition to diabetes or of diagnosing a susceptibility to diabetes comprising detecting a polymorphism in the ESE-1 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-1 gene, wherein detection of said polymorphism is indicative of the occurrence of diabetes or a predisposition to diabetes or a susceptibility to diabetes.

8. The method of Claim 6 or 7 wherein said polymorphism is selected from the group consisting of: a SNP at position −2034, a SNP at position +171, a SNP at position +949, a SNP at position +1275, a SNP at position +1639, a SNP at position +1744 and a SNP at position +2836.

9. The method of Claim 6 or 7 wherein said polymorphism is selected from the group consisting of:

a) a SNP at position -2034 and said SNP is a cytosine (C) nucleotide in comparison to a adenine (A) nucleotide in the wildtype ESE-1 gene;

b) a SNP at position +171 and said SNP is a cytosine (C) nucleotide in comparison to a thymine (T) nucleotide in the wildtype ESE-1 gene;

c) a SNP at position +949 and said SNP is a thymine (T) nucleotide in comparison to a guanine (G) nucleotide in the wildtype ESE-1 gene;

d) a SNP at position +1275 and said SNP is an adenine (A) nucleotide in comparison to a cytosine (C) nucleotide in the wildtype ESE-1 gene;

e) a SNP at position +1639 and said SNP is an adenine (A) nucleotide in comparison to a guanine (G) nucleotide in the wildtype ESE-1 gene; and

f) a SNP at position +1744 and said SNP is an adenine (A) nucleotide in comparison to a guanine (G) nucleotide in the wildtype ESE-1 gene.

10. A method of diagnosis of autoimmune disease or of a predisposition to autoimmune disease or of diagnosing a susceptibility to autoimmune disease comprising detecting a polymorphism in the ESE-2 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-2 gene, wherein detection of said polymorphism is indicative of the occurrence of autoimmune disease or a predisposition to autoimmune disease or a susceptibility to autoimmune disease.

11. A method of diagnosis of diabetes or of a predisposition to diabetes or of diagnosing a susceptibility to diabetes comprising detecting a polymorphism in the ESE-2 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-2 gene, wherein detection of said polymorphism is indicative of the occurrence of diabetes or a predisposition to diabetes or a susceptibility to diabetes.

12. The method of Claim 10 or 11 wherein said polymorphism is a SNP at position -151 and said SNP is a cytosine (C) nucleotide in comparison to an guanine (G) nucleotide in the wildtype ESE-2 gene.

13. The method of any one of Claim 1, 6 or 10 wherein said autoimmune disease is selected from the group consisting of: diabetes, multiple sclerosis, rheumatoid arthritis, lupus, psoriasis, asthma, myasthenia gravis, Sjogrens syndrome, Hashimoto's thyroiditis and Pemphigus vulgaris.

14. The method of any one of Claim 2, 7 or 11 wherein said diabetes is selected from the group consisting of: Type I diabetes and Type II diabetes.

15. A method of treating autoimmune disease in an individual comprising the steps of:

a) screening an individual for a genetic predisposition to autoimmune disease by detecting the presence of a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene; and

b) if such a predisposition is identified, treating the individual to prevent or reduce autoimmune disease or to delay the onset of autoimmune disease.

16. A method of treating diabetes in an individual comprising the steps of:

a) screening an individual for a genetic predisposition to diabetes by detecting the presence of a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene; and

b) if such a predisposition is identified, treating the individual to prevent or reduce diabetes or to delay the onset of diabetes.

17. A method of treating multiple sclerosis in an individual comprising the steps of:

a) screening an individual for a genetic predisposition to multiple sclerosis by detecting the presence of a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene; and

b) if such a predisposition is identified, treating the individual to prevent or reduce multiple sclerosis or to delay the onset of multiple sclerosis.

18. The method of any one of Claim 15, 16 or 17 wherein said polymorphism is selected from the group consisting of:

a) a SNP at position -140 and said SNP is a guanine (G) nucleotide in comparison to an adenine (A) nucleotide in the wildtype ESE-3 gene; and

b) a SNP at position -4458 and said SNP is a thymine (T) nucleotide in comparison to a cytosine (C) nucleotide in the wildtype ESE-3 gene.

19. A method of treating autoimmune disease in an individual comprising the steps of:

a) screening an individual for a genetic predisposition to autoimmune disease by detecting the presence of a polymorphism in the ESE-1 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-1 gene; and

b) if such a predisposition is identified, treating the individual to prevent or reduce autoimmune disease or to delay the onset of autoimmune disease.

20. A method of treating diabetes in an individual comprising the steps of:

a) screening an individual for a genetic predisposition to diabetes by detecting the presence of a polymorphism in the ESE-1 gene which is

correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-1 gene; and

b) if such a predisposition is identified, treating the individual to prevent or reduce diabetes or to delay the onset of diabetes.

21. A method of treating autoimmune disease in an individual comprising the steps of:

a) screening an individual for a genetic predisposition to autoimmune disease by detecting the presence of a polymorphism in the ESE-2 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-2 gene; and

b) if such a predisposition is identified, treating the individual to prevent or reduce autoimmune disease or to delay the onset of autoimmune disease.

22. A method of treating diabetes in an individual comprising the steps of:

a) screening an individual for a genetic predisposition to diabetes by detecting the presence of a polymorphism in the ESE-2 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-2 gene; and

b) if such a predisposition is identified, treating the individual to prevent or reduce diabetes or to delay the onset of diabetes.

23. The method of any one of Claim 15, 19 or 21 wherein said autoimmune disease is selected from the group consisting of: diabetes, multiple sclerosis, rheumatoid arthritis, lupus, psoriasis, asthma, myasthenia gravis, Sjogrens syndrome, Hashimoto's thyroiditis and Pemphigus vulgaris.

24. The method of any one of Claim 16, 20 or 22 wherein said diabetes is selected from the group consisting of: Type I diabetes and Type II diabetes.

25. A method of treating or preventing autoimmune disease in an individual having a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene comprising administering to said individual an agent that interferes with the function or expression of ESE-3.

26. A method of treating or preventing diabetes in an individual having a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene comprising administering to said individual an agent that interferes with the function or expression of ESE-3.

27. A method of treating or preventing inflammation in an individual having a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene comprising administering to said individual an agent that interferes with the function or expression of ESE-3.

28. The method of Claim 27 wherein said inflammation is associated with an inflammatory disease selected from the group consisting of: atherosclerosis and rheumatoid arthritis.

29. The method of Claim 27 wherein said inflammation is localized inflammation associated with restenosis.

30. A method of treating or preventing multiple sclerosis in an individual having a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene comprising administering to said individual an agent that interferes with the function or expression of ESE-3.

31. The method of any one of Claim 25 to 30 wherein said polymorphism is selected from the group consisting of:

a) a SNP at position -140 and said SNP is a guanine (G) nucleotide in comparison to an adenine (A) nucleotide in the wildtype ESE-3 gene; and

b) a SNP at position -4458 and said SNP is a thymine (T) nucleotide in comparison to a cytosine (C) nucleotide in the wildtype ESE-3 gene.

32. The method of any one of Claim 25 to 31 wherein said agent blocks the function or expression of ESE-3.

33. A method of assaying a sample for the presence of a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene comprising contacting said sample with an antibody which specifically binds to the encoded polypeptide.

34. A reagent kit for assaying a sample for the presence of a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide which is encoded by said ESE-3 gene comprising in separate containers:

a) one or more labeled antibodies which specifically binds to said polypeptide, and

a) reagents for detecting said label

35. A method of assaying a sample for the presence of a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene comprising contacting such gene with a nucleotide comprising:

a) contacting said sample with a nucleic acid comprising a contiguous nucleotide sequence which is at least partially complementary to the ESE-3 gene under conditions appropriate for hybridization, and

b) assessing whether hybridization has occurred between an ESE-3 gene and said nucleic acid comprising a contiguous nucleotide sequence which is at least partially complementary to the ESE-3 gene

36. The method of Claim 35 wherein said nucleic acid comprising a contiguous nucleotide sequence is completely complementary to said ESE-3 gene nucleic acid.

37. The method of Claim 35 comprising amplification of said ESE-3 gene nucleic acid.

38. A reagent kit for assaying a sample for the presence of a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide which is encoded by said ESE-3 gene comprising in separate containers:

a) one or more labeled nucleic acids comprising a contiguous nucleic acid sequence which is at least partially complementary to a part of said ESE-3 nucleotide sequence, and

b) reagents for detecting said label.

39. A method of interfering with the function or expression of ESE-3 in an individual having a polymorphism in the ESE-3 gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene comprising administering to said individual an agent that interferes with the function or expression of ESE-3.

40. A method of identifying an agent which interferes with the function or expression of ESE-3 in an individual having a polymorphism in the ESE-3

gene which is correlated with an alteration in the activity or expression of a polypeptide encoded by said ESE-3 gene comprising:

a) contacting said ESE-3 gene with the agent to be tested, and

b) assessing the level of function or expression of said ESE-3 gene, and

c) comparing the level of function or expression with the level of function or expression of said ESE-3 gene in the absence of the agent.

SNPs of Potential Functional Significance in Type I diabetes and other autoimmune diseases

ESE-3	-140		
WT:	5′ Cy3-AAT GAG CCA	A-3′	
Mutant:	5′ Cy5-AAT GAG CCA	G-3′	
ESE-2a	-151 (SNP Id #836146)		
WT:	5′ Cy3-AGC AAT AAC A	G-3′	
Mutant:	5′ Cy5-AGC AAT AAC A	C-3′	
ESE-1	+171 (SNP Id #1135541)		
WT:	5′ Cy3-AGA GAA GGC	T-3′	
Mutant:	5′ Cy5-AGA GAA GGC	C-3′	
ESE-1	+949 (SNP Id #1135542)		
WT:	5′ Cy3-TCT TTT TCT TTT	G-3′	
Mutant:	5′ Cy5-TCT TTT TCT TTT	T-3′	

ESE-1	+1275 (SNP Id#13690)		
WT:	5′ Cy3-CCG AGT CC	C-3′	
Mutant:	5′ Cy5-CCG AGT CC	A-3′	
ESE-1	+1639 (SNP Id#1135680)		
WT:	5′ Cy3-GGA ATG TTT TA	G-3′	
Mutant:	5′ Cy5-GGA ATG TTT TA	A-3′	
ESE-1	+1744 (SNP Id#1135680)		
WT:	5′ Cy3-ATT CAC AGG A	G-3′	
Mutant:	5′ Cy5-ATT CAC AGG A	A-3′	

Fig. 1A

Genbank Accession No	SNP ID				WT	SNP
	rs2735765	-2034	ESE-1, Ets family transcription factor	AACAAAATAAAC[A/C]AAATACTAGATCTGGAA	A	C
NT004612		+171	ESE-1, Ets family transcription factor	TGCAGAGAAGGC[C/T]AGCTGGTTGGGGGAA	C	T
NT004612		+949	ESE-1, Ets family transcription factor	CAACTATGGGGC[C/A]AAAAGAAAAAGAACAG	C	A
	rs11135542	2836	ESE-1, Ets family transcription factor	CAACTATGGGGC[A/C]AAAAGAAAAAGAACAG	A	C
	rs475043	far 3'UTR	ESE-2, ELF5, E74-like factor 5 (ets domain transcription factor)	AATAGCAAATGCCC[C/T]AACTTTGAAAATTACTTA	C	T
	rs838143	-257	ESE-2, ELF5, E74-like factor 5 (ets domain transcription factor)	GGGATCGTGCGTTTG[C/G]TGAGGAATTTCCTCT	C	G
G9787a0		361	ESE-2, ELF5, E74-like factor 5 (ets domain transcription factor)	GTAGGTGACACTAGG[C/G]GGTGCCTGCTCCTAG	C	G
G9787a1		845	ESE-2, ELF5, E74-like factor 5 (ets domain transcription factor)	ATTTGAAGGAGCCCAT[C/G]CTGAGTGCAGCCAGG	T	C
G9787a2		12789	ESE-2, ELF5, E74-like factor 5 (ets domain transcription factor)	TCAGTGTTCAGAGGG[C/A]CAGACCTCCAGGGGA	C	A
G9787a3		12857	ESE-2, ELF5, E74-like factor 5 (ets domain transcription factor)	TTTCCCATCGTGTGA[A/C]TTGCACAGCAAAATT	A	C
G9787a4		15950	ESE-2, ELF5, E74-like factor 5 (ets domain transcription factor)	AATCTGCAGAGGCTG[C/T]TGGGAAGCCTGGTTC	C	T
G9787a5		16257	ESE-2, ELF5, E74-like factor 5 (ets domain transcription factor)	TGTAAGTAACCAAGC[G/T]CAAGCCACCCAAGGC	G	T
G9787a8		25974	ESE-2, ELF5, E74-like factor 5 (ets domain transcription factor)	TTTGGAGCGGGTTGA[C/T]CGAAGGTTAGTGTAC	C	T
AF124439		9365	ESE-3, EHF, Ets family transcription factor	TCTCCCTGGGGAAAA[T/A]GATCGCTGACTATTC	T	A
AF124439		9554	ESE-3, EHF, Ets family transcription factor	ATTGTGAGGTGGGGG[G/T]AGGGGGTGCTCTAAA	G	T
AF124439		16693	ESE-3, EHF, Ets family transcription factor	CTACAAAAGAGAAAT[A/T]CTGGAGCGTGTGGAT	A	T
AF124439		16346	ESE-3, EHF, Ets family transcription factor	TGACTTAGATCATTA[G/A]TAACCTGCCTTTCTG	G	A
AF124439		4458	ESE-3, EHF, Ets family transcription factor	CAACGGCGAGCACCT[C/T]TGCAGCATGAGTTTG	C	T
AF124439		14728	ESE-3, EHF, Ets family transcription factor	AATAGGTCAATTAAAT[T/C]GGCTCTCATTTTCT	T	C
AF124439		14728	ESE-3, EHF, Ets family transcription factor	TAGGTCAATTAAATG[G/C]CTCTCATTTTCTCC	G	C
AF124439		15095	ESE-3, EHF, Ets family transcription factor	TGCAGGCATGGGGTT[A/G]GCAATTTCCATCTCT	A	G
	rs288927	-1079	ESE-3, EHF, Ets family transcription factor	AAGGTTCAACAGAGA[A/G]AGTTAATGGTTGGG	A	G
AF163685		-140	ESE-3, EHF, Ets family transcription factor	GCAAATGAGCCA[A/G]TGTTTGTAATCCTTTATT	A	G
	rs286925	-9	ESE-3, EHF, Ets family transcription factor	GCTCAGACTTGATAAC[A/G]CCCGTGGTGCCCCA	A	G
	rs704733	far 3'UTR	ESE-3, EHF, Ets family transcription factor	TCATGAAAAAGCAC[C/G]AGGTTCTAAGTGG	C	G
	rs447228	7-21	ESE-3, EHF, Ets family transcription factor	AATAGGTCAATTAAA[C/T]GNCTCTCATTTTCTC	C	T
AF279459		-308	Tumor Necrosis Factor (TNF) α	TTGAGGGGCATG[G/A]GGACGGGGTTCAG	G	A
AY070490		+249	Lymphotoxin (TNF-β)	GTTTCTGCCATG[A/G]TTCCTCTCTGTTCCC	A	G
AY070490		+365	Lymphotoxin (TNF-β)	CGCCCTGGGAGA[C/G]AGCACCCCCCGA	C	G
AY070490		+720	Lymphotoxin (TNF-β)	TTGCCCACAGCA[C/A]CCTCAAACCTGC	C	A
		+869	Transforming Growth Factor β1 (TGF-β)	CTGCGGCTGCTGCC[C/T]GCTGCTGCTACCGC	C	T
		+915	Transforming Growth Factor β1 (TGF-β)	TGACGCCTGGCC[G/C]GCCGGCCGCGGGAC	G	C
		-1141	Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4)	GGGAAACCATGGA[C/T]GGACTGGAGTAGG	C	T
AF142144		-651	Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4)	GCTTCTTTTC[C/T]GCCTATTTTCAGTTTATTT	C	T
AF142144		-318	Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4)	TTATCCAGATCCT[C/T]AAAGTGAACATGAAG	C	T
AF142144	rs231775	+49	Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4)	CTGAACCTGGCT[A/G]CCAGGACCTGGCCCT	A	G
AF142144		+159	Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4)		C	G
		-174	IL-8	GTTGTGTCTTGC[G/C]ATGCTAAAGGACGTCA	G	C
		-1082	IL-10	GGCTTCTTTGGGA[G/A]GGGGAAGTAGGGAT	G	A
		-819	IL-10	CAGGTGATGTAA[C/T]ATCTCTGTGCCTCAGTT	C	T
		-592	IL-10	TGACCCCGCCTGT[C/A]CTGTAGGAAGCCA	C	A
	rs2233642	3279 aa14 PDEF		GGCTCCAGTCCA[C/T]GGGATCTGGGCAAGA	C	T

Fig. 1B

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-500  CAAAGCAGGGTGGACTAAATACAGACTAATAAATGAGACAGGTGCTCAAGAGGGCCCCTCCATACCATCATCTCCTCCGGATTTGGACTTCTACTCACTT  -401

-400  TGCTTTTACATTCCCTCTTCCCGATGGTGTCTTTGGTGAGCAGGGTGCTTTTCACCTGAAACAGCCTCTGAGCTGAAAAGAACAGTCACCACCAAATCAA  -301
                                                                          HLH
-300  TTCCTCATCCATTAACAGGTTGTCTCTCTGTTCTTGAGACACAGGCATTACCTGGTTAGACCTGTTTTGTTTGAACACTAACGTGTGAGTTGGCCAAATG  -201
                                                                                                      OCT
                G
-200  CAAATGAGCCAATGTTTGTAATCCTTTATTTTATTTTTTTAAAGGGCTGGGTAGCCAATCAGAAGAGGGGGAAGTGACTTAGGGAATTCCCGGTTGGTGG  -101

       OCT     NF-Y                                                        NF-Y                 AP-1     NF-kB
-100  CTTATTGCTTAACATCCTACAAAATGATTTAAAATTATTGTTATATGCATTTATCTTCACTCTGATGAGGGCTCAGACTTGATAACGCCCGTGGTGCCCC  -1
                                  SOX5                                                            GATA
   1  ATCCCTATAGGAGCTGGTGAGATTGCAGCCTGCTGCCTCCCCTCCATCAGCCACAGCTATTGGATTTCCCACCCAGAATCTTTAGGTAAATGAG      94
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Fig. 2

			Bahrein patients				
samples	Patient	TNFa (-308)	LT (249) lymphotoxin	ESE-3 (-140)	CTLA4 (+49)		
1	Type I Diabetes	G/G	A/G	A/A	A/A		
2	Type I Diabetes	G/A	G/G	A/A	A/G		
3	Type I Diabetes	G/G	A/A	A/A	A/G		
4	Type I Diabetes	G/G	A/A	A/A	A/A		
5	Type I Diabetes	G/G	A/A	A/G	A/A		
6	Type I Diabetes	G/G	A/A	A/A	A/G		
7	Type I Diabetes	G/G	A/G	A/G	A/A		
8	Type I Diabetes	G/A	G/G	A/G	A/A		
9	Type I Diabetes	G/G	G/G	A/A	A/A		
10	Type I Diabetes	G/G	A/A	A/A	A/A		
11	Type I Diabetes	G/G	A/G	A/A	A/G		
12	Type I Diabetes	G/A	A/G	A/G	A/A		
13	Type I Diabetes	G/G	A/G	A/G	A/A		
14	Type I Diabetes	G/G	A/G	A/A	A/A		
15	Type I Diabetes	G/G	A/A	A/A	A/A		
16	Type I Diabetes	G/G	A/A	A/A	A/A		
17	Type I Diabetes	G/G	A/G	A/A	A/G		
18	Type I Diabetes	G/G	A/A	A/A	G/G		
19	Type I Diabetes	G/G	A/G	A/A	A/G		
20	Type I Diabetes	G/G	A/G	A/A	A/A		
21	Type I Diabetes	G/A	G/G	A/A	A/A		
22	Type I Diabetes	G/G	A/G	A/A	A/A		
23	Type I Diabetes	G/A	A/A	A/A	G/G		
24	Type I Diabetes	G/G	A/A	A/A	A/G		
25	Type I Diabetes	G/G	G/G	A/A	A/G		
26	Type I Diabetes	G/G	A/G	A/A	A/G		
27	Type I Diabetes	G/G	A/G	A/G	A/A		
28	Type I Diabetes	G/G	A/A	A/A	A/G		
29	Type I Diabetes	G/G	A/G	A/G	A/G		
30	Type I Diabetes	G/G	A/A	A/G	A/G		
31	Type I Diabetes	G/G	A/A	A/A	A/A		
32	Type I Diabetes	G/A	A/G	A/A	A/A		
33	Type I Diabetes	G/A	A/A	A/G	A/G		
34	Type I Diabetes	G/A	G/G	A/G	A/G		
35	Type I Diabetes	G/G	A/G	A/G	A/A		
36	Type I Diabetes	G/G	A/A	A/A	A/A		
37	Type I Diabetes	G/G	A/A	A/A	A/G		
38	Type I Diabetes	G/G	A/A	A/A	A/G		
39	Type I Diabetes	G/A	A/G	A/A	A/A		

Fig. 3A

40	Type I Diabetes	G/G	A/A	G/G	A/A		
41	Type I Diabetes	G/G	A/A	A/G	A/G		
42	Type I Diabetes	G/G	A/A	A/A	A/G		
43	Type I Diabetes	A/A	G/G	A/G	A/G		
44	Type I Diabetes	G/G	G/G	A/A	A/A		
45	Type I Diabetes	G/G	A/A	A/G	A/G		
46	Type I Diabetes	G/G	A/A	A/G	A/G		
47	Type I Diabetes	G/G	A/A	A/G	A/G		
48	Type I Diabetes	G/G	A/G	A/G	A/A		
49	Type I Diabetes	G/G	A/G	A/A	A/G		
50	Type I Diabetes	G/A	G/G	A/G	A/A		
51	Type I Diabetes	G/A	A/G	A/G	A/A (3:1)		
52	Type I Diabetes	G/G	A/G	A/G	A/A (4:1)		
53	Type I Diabetes	G/G	A/G	A/G	A/A		
54	Type I Diabetes	G/G	G/G	A/G	A/G		
55	Type I Diabetes	G/G	G/G	A/A	A/A		
56	Type I Diabetes	G/G	A/G	A/A	A/G		
57	Type I Diabetes	G/G	G/G	A/G	A/G		
58	Type I Diabetes	G/A	A/G				
59	Type I Diabetes	G/A	G/G	A/G	A/G		
60	Type I Diabetes	A/A	G/G	A/G	A/G		
61	Type I Diabetes	G/A	A/G	A/G	A/G		
62	Type I Diabetes	G/G	A/G	A/A	A/G		
63							
64	Type II Diabetes	G/G	A/A	A/A			
65	Type II Diabetes	G/G	A/A	A/A	A/A		
66	Type II Diabetes	G/G	A/A	A/A			
67	Type II Diabetes	G/G			A/A		
68	Type II Diabetes	G/G	A/A				
69	Type II Diabetes	G/G			A/G		
70	Type II Diabetes	G/G	A/A		A/G		
71	Type II Diabetes						
72	Type II Diabetes	G/G		A/G			
73	Type II Diabetes	G/G	A/A	A/A	G/G		
74	Type II Diabetes	G/G	A/A	A/A	A/A		
75	Type II Diabetes		A/G		A/G		
76	Type II Diabetes	G/G			A/A		
77	Type II Diabetes	G/G					
78	Type II Diabetes	G/G	A/A	G/G			
79	Type II Diabetes						
80	Type II Diabetes		A/G	A/G			
81	Type II Diabetes	G/G	A/A	A/A			
82	Type II Diabetes	G/G	A/A	A/A	A/A		
83	Type II Diabetes	G/G	A/A	A/A			
84	Type II Diabetes	G/G		A/G	A/A		
85	Type II Diabetes	G/G	A/A	A/A			

Fig. 3B

86	Type II Diabetes	G/G	A/A	A/A	A/A		
87	Type II Diabetes	G/G	A/A	A/A	A/A		
88	Type II Diabetes	G/G	G/G				
89	Type II Diabetes	G/G	A/A	A/A			
90	Type II Diabetes	G/G	A/A	A/A	A/A		
91	Type II Diabetes	G/G			A/A		
92	Type II Diabetes	G/G		A/G			
93	Type II Diabetes	G/G	G/G	A/G			
94	Type II Diabetes	G/G					
95	Type II Diabetes						
96	Type II Diabetes	G/G			G/G		
97	Type II Diabetes	G/G			A/A		
98	Type II Diabetes	G/G		A/G			
99	Type II Diabetes	G/G			A/A		
100	Type II Diabetes		G/G	A/A	A/G		
101	Type II Diabetes		A/G				
102	Type II Diabetes	G/G	A/A		A/G		
103	Control	G/G	A/A	A/A	A/A		
104	Control	G/G	A/A	A/A	A/A		
105	Control	G/G	A/A	A/A	A/G		
106	Control	G/A	G/G	A/A	A/G		
107	Control	G/G	A/A	A/A	A/A		
108	Control	G/G	A/A	A/A	A/G		
109	Control	G/G	A/G	A/A	A/G		
110	Control	G/G	A/A	A/A	A/G		
111	Control	A/A	G/G	A/A	A/A		
112	Control	G/G	A/G	G/G	A/G		
113	Control	G/A	A/G	A/A	A/G		
114	Control	G/G	A/A	A/A	A/A		
115	Control	G/G	A/A	A/A	A/G		
116	Control	G/G	A/A	G/G	A/A		
117	Control	G/G	G/G	A/A	A/A		
118	Control	G/G	G/G	A/G	A/A		
119	Control	G/G	A/G	A/A	A/A		
120	Control	G/G	A/A	A/G	A/A		
121	Control	G/G	A/A	A/A	A/G		
122	Control	G/G	A/A	A/A	A/A		
123	Control	G/G	A/G	A/A	A/G		
124	Control	G/A	A/G	A/G	A/A		
125	Control	G/G	A/A	A/A	A/A		
126	Control	G/A	A/G	A/A	A/A		
127	Control	G/G	A/A	A/A	A/A		
128	Control	G/G	A/A	A/G	A/G		
129	Control	G/G	A/G	A/A	A/G		

Fig. 3C

131	Control	G/G	A/G	A/A	A/G		
132	Control	G/G	A/A	A/A	A/A		
133	Control	G/A	A/G	A/G			
134	Control	G/G	A/A	A/A	A/A		
135	Control	G/G	A/A	A/G	A/G		
136	Control	G/A	G/G	A/A	A/A		
137	Control	G/G	A/G	A/A	A/G		
138	Control	G/G	A/A	A/A	A/G		
139	Control	G/G	A/G	A/G	A/G		
140	Control	G/G	A/G	A/G	A/G		
141	Control	G/A	A/G	A/A	A/A		
142	Control	G/G	A/A	A/G	A/G		
143	Control	G/G	A/G	A/G	A/G		
144	Control	G/G	A/A	A/A	A/G		
145	Control	G/G	A/A	A/A	A/A		
146	Control	G/G	A/A	A/G	A/G		
147	Control	A/A	G/G	A/A	A/A		
148	Control	G/G	A/G	A/A	A/A		
149	Control	G/G	A/G		A/G		
150	Control	G/G	A/A	A/A	A/G		
		TNFa (-308)	LT (249)	ESE-3	CTLA-4 (+49)		
WT/WT	Type I Diabetes	46	23	A/A 34	A/A 29		
WT/SNP	Type I Diabetes	14	25	A/G 26	A/G 30		
SNP/SNP	Type I Diabetes	2	14	G/G 1	G/G 2		
WT/WT	Type II Diabetes	G/G 34	18	A/A 27	A/A 18		
WT/SNP	Type II Diabetes	G/A 4	18	A/G 9	A/G 17		
SNP/SNP	Type II Diabetes	A/A 0	3	G/G 1	G/G 2		
WT/WT	Control	39	25	A/A 34	A/A 23		
WT/SNP	Control	7	17	A/G 11	A/G 24		
SNP/SNP	Control	2	6	G/G 2	G/G 0		
WT		74%	37%	56%	48%		
SNP		26%	63%	44%	52%		
WT		89%	46%				
SNP		11%	54%				
WT		81%	52%	72%	51%		
SNP		19%	48%	28%	49%		

Fig. 3D

Associations: Type II
Diabetes (NIDD) vs. Control

ESE-3

	A/A	A/G	G/G
IDD	53	41	1
NIDD	42	23	1
CTRL	42	15	2

TNF-a

	A/A	A/G	G/G
IDD	3	22	74
NIDD	2	14	53
CTRL	2	18	61

LT 249

	A/A	A/G	G/G
IDD	23	25	14
NIDD	18	18	3
CTRL	25	17	6

CTLA4

	A/A	A/G	G/G
IDD	48	49	3
NIDD	49	46	5
CTRL	49	51	0

Fig. 4A

ESR-3/IDD					TER			X2	
	WT		Mut			WT	Mut		3.0
IDD		53	42	95m1	IDD	58.6	36.4	p val	
CTRL		42	17	59m2	CTRL	36.4	22.6	0.0082047	86
		95	59	154					
	n1		n2						

TNF-a/IDD					TER			X2	
	WT		Mut			WT	Mut		0.0
IDD		74	25	99m1	IDD	74.3	24.7	p val	
CTRL		61	20	81m2	CTRL	60.8	20.3	0.0054913	77
		135	45	180					
	n1		n2						

LT249/IDD					TER			X2	
	WT		Mut			WT	Mut		1.9
IDD		23	39	62m1	IDD	27.1	34.9	p val	
CTRL		25	23	48m2	CTRL	21.0	27.1	0.0078074	
		48	62	110					
	n1		n2						

CTLA4/IDD					TER			X2	
	WT		Mut			WT	Mut		0.0
IDD		48	52	100m1	IDD	48.5	51.5	p val	
CTRL		49	51	100m2	CTRL	48.5	51.5	0.0	
		97	103	200					
	n1		n2						

Fig. 4B

Associations: Type II Diabetes (NIDD) vs. Control

ESE-3

	A/A	A/G	G/G
IDD	53	41	1
NIDD	42	23	1
CTRL	42	15	2

TNF-a

	A/A	A/G	G/G
IDD	3	22	74
NIDD	2	14	53
CTRL	2	18	61

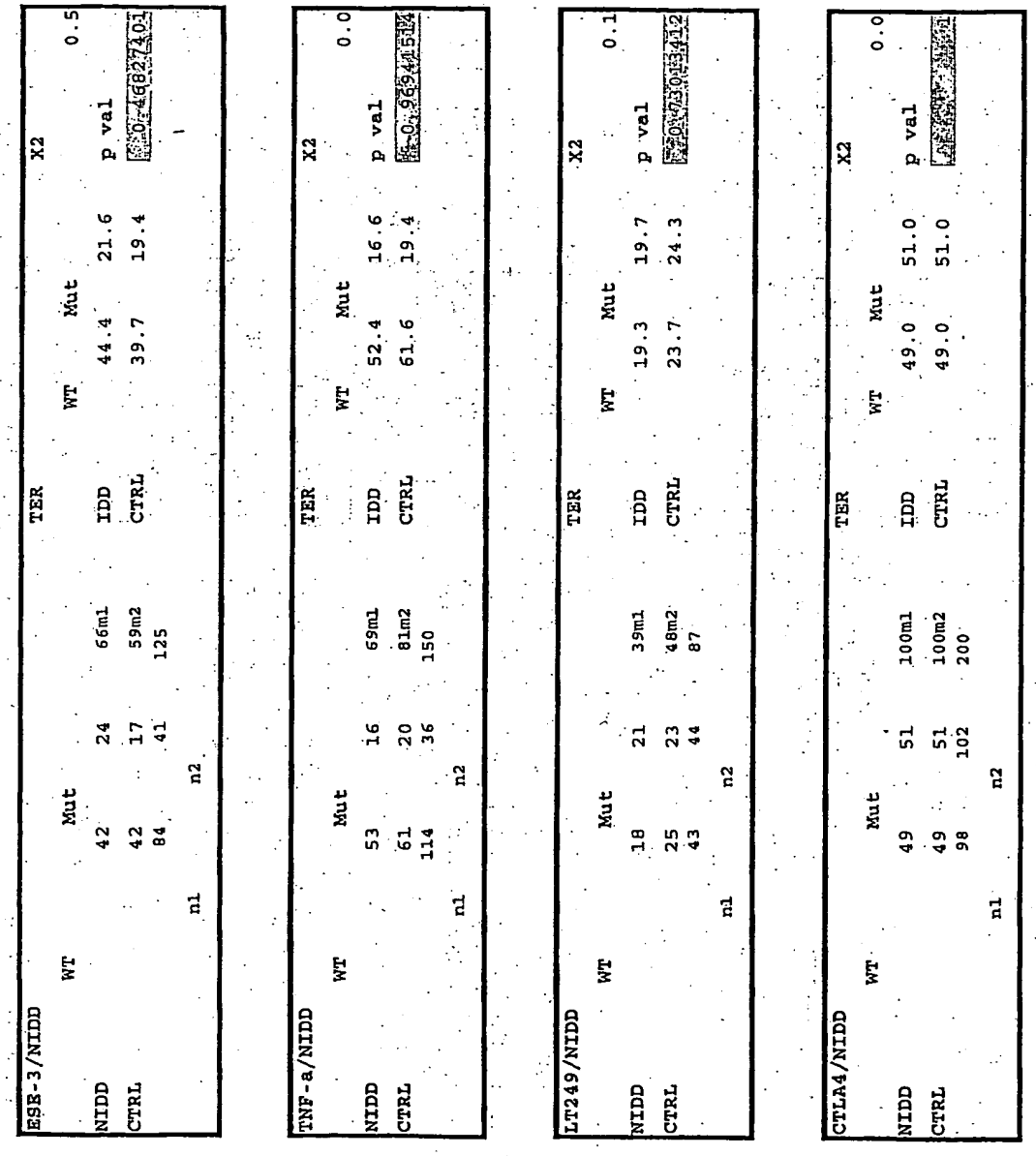
LT 249

	A/A	A/G	G/G
IDD	23	25	14
NIDD	18	18	3
CTRL	25	17	6

CTLA4

	A/A	A/G	G/G
IDD	48	49	3
NIDD	49	46	5
CTRL	49	51	0

Fig. 5A



ESR-3/NIDD				TER			X2	0.5
	WT		Mut		WT	Mut		
NIDD		42	24	66m1	IDD	44.4	21.6	p val
CTRL		42	17	59m2	CTRL	39.7	19.4	0.468271
		84	41	125				
	n1		n2					

TNF-a/NIDD				TER			X2	0.0
	WT		Mut		WT	Mut		
NIDD		53	16	69m1	IDD	52.4	16.6	p val
CTRL		61	20	81m2	CTRL	61.6	19.4	0.994151
		114	36	150				
	n1		n2					

LT249/NIDD				TER			X2	0.1
	WT		Mut		WT	Mut		
NIDD		18	21	39m1	IDD	19.3	19.7	p val
CTRL		25	23	48m2	CTRL	23.7	24.3	0.074013
		43	44	87				
	n1		n2					

CTLA4/NIDD				TER			X2	0.0
	WT		Mut		WT	Mut		
NIDD		49	51	100m1	IDD	49.0	51.0	p val
CTRL		49	51	100m2	CTRL	49.0	51.0	[illegible]
		98	102	200				
	n1		n2					

Fig. 5B

						Bahrein patients					
sample id	key	plate	original name	subgroup	TNFa (-308)	LT (249)	ESE3 (-140)	CTLA4 (+49)	TGFb (25)	Il-6P	ESE3 (-4458)
1	BH	1	1	IDD	G/G	A/G	A/A	A/A	G/G	G/G	
2	BH	1	2	IDD	G/A	G/G	A/A	A/G	G/G	G/G	G/A
3	BH	1	3	IDD	G/G	A/A	A/A	A/G	G/G	G/G	G/A
4	BH	1	4	IDD	G/G	A/A	A/A	A/A	G/G	G/G	G/G
5	BH	1	5	IDD	G/G	A/A	A/G	A/A	G/G		G/G
6	BH	1	6	IDD	G/G	A/A	A/A	A/G	G/G	G/C	G/G
7	BH	1	7	IDD	G/G	A/G	A/G	A/A	G/G	G/G	G/G
8	BH	1	8	IDD	G/A	G/G	A/G	A/A	G/G	G/G	G/G
9	BH	1	9	IDD	G/G	G/G	A/A	A/A	G/G		G/G
10	BH	1	10	IDD	G/G	A/A	A/A	A/A	G/G		G/G
11	BH	1	11	IDD	G/G	A/G	A/A	A/G	G/G	G/G	G/G
12	BH	1	12	IDD	G/A	A/G	A/G	A/A	G/C	G/G	G/G
13	BH	1	13	IDD	G/G	A/G	A/G	A/A	G/C	G/G	G/G
14	BH	1	14	IDD	G/G	A/G	A/A	A/A	G/G	G/C	G/G
15	BH	1	15	IDD	G/G	A/A	A/A	A/A	G/G	G/C	G/G
16	BH	1	16	IDD	G/G	A/A	A/A	A/A	G/G	G/C	G/G
17	BH	1	17	IDD	G/G	A/G	A/A	A/G	G/G	G/C	G/G
18	BH	1	18	IDD	G/G	A/A	A/A	G/G	G/G	G/G	G/A
19	BH	1	19	IDD	G/G	A/G	A/A	A/G	G/G	G/C	G/A
20	BH	1	20	IDD	G/G	A/G	A/A	A/A	G/G	G/G	G/G
21	BH	1	21	IDD	G/A	G/G	A/A	A/A	G/G	G/C	G/G
22	BH	1	22	IDD	G/G	A/G	A/A	A/A	G/G	G/G	G/G
23	BH	1	23	IDD	G/A	A/A	A/A	G/G	G/G	G/G	G/G
24	BH	1	24	IDD	G/G	A/A	A/A	A/G	G/G	G/C	G/G
25	BH	1	25	IDD	G/G	G/G	A/A	A/G	G/G	G/G	G/G
26	BH	1	26	IDD	G/G	A/G	A/A	A/G	G/G	G/G	G/G
27	BH	1	27	IDD	G/G	A/G	A/G	A/A	G/G	G/G	G/G
28	BH	1	28	IDD	G/G	A/A	A/A	A/G	G/C	G/G	G/G
29	BH	1	29	IDD	G/G	A/G	A/G	A/G	G/C	G/C	G/G
30	BH	1	30	IDD	G/G	A/A	A/G	A/G	G/G	G/G	G/G
31	BH	1	31	IDD	G/G	A/A	A/A	A/A	G/G	G/G	G/G
32	BH	1	32	IDD	G/A	A/G	A/A	A/A	G/G	G/C	G/A
33	BH	1	33	IDD	G/A	A/A	A/G	A/G	G/C	G/G	G/G
34	BH	1	34	IDD	G/A	G/G	A/G	A/G	G/G	G/G	G/G
35	BH	1	35	IDD	G/G	A/G	A/G	A/A	G/C	G/G	G/A
36	BH	1	36	IDD	G/G	A/A	A/A	A/A	G/C	G/G	G/A
37	BH	1	37	IDD	G/G	A/A	A/A	A/G	G/G	G/G	G/G
38	BH	1	38	IDD	G/G	A/A	A/A	A/G	G/C		G/G
39	BH	1	39	IDD	G/A	A/G	A/A	A/A	G/G	G/G	G/A
40	BH	1	40	IDD	G/G	A/A	G/G	A/A	G/C	G/G	G/G
41	BH	1	41	IDD	G/G	A/A	A/G	A/G	G/G	G/G	G/G
42	BH	1	42	IDD	G/G	A/A	A/A	A/G	G/G	G/G	G/G
43	BH	1	43	IDD	A/A	G/G	A/G	A/G	G/G	G/G	G/G
44	BH	1	44	IDD	G/G	G/G	A/A	A/A	G/G	G/C	G/G
45	BH	1	45	IDD	G/G	A/A	A/G	A/G	G/G	G/G	G/A
46	BH	1	46	IDD	G/G	A/A	A/G	A/G	G/G	G/G	G/G
47	BH	1	47	IDD	G/G	A/A	A/G	A/G	G/G	G/C	G/G
48	BH	1	48	IDD	G/G	A/G	A/G	A/A	G/C	G/G	G/A
49	BH	1	49	IDD	G/G	A/G	A/A	A/G	G/G	G/C	G/A
50	BH	1	50	IDD	G/A	G/G	A/G	A/A	G/G	G/C	G/G
51	BH	1	51	IDD	G/A	A/G	A/G	A/A	G/G	G/C	
52	BH	1	52	IDD	G/G	A/G	A/G	A/A	G/G	G/G	A/A
53	BH	1	53	IDD	G/G	A/G	A/G	A/A	G/G	G/G	A/A
54	BH	1	54	IDD	G/G	G/G	A/G	A/G	G/G	G/C	G/A
55	BH	1	55	IDD	G/G	G/G	A/A	A/A	G/G	G/G	G/G
56	BH	1	56	IDD	G/G	A/G	A/A	A/G	G/G	G/G	
57	BH	1	57	IDD	G/G	G/G	A/G	A/G	G/G	G/G	G/G
58	BH	1	58	IDD	G/A	A/G			G/G		G/G
59	BH	1	59	IDD	G/A	G/G	A/G	A/G	G/G	G/C	G/A
60	BH	1	60	IDD	A/A	G/G	A/G	A/G	G/G	G/G	G/G
61	BH	1	61	IDD	G/A	A/G	A/G	A/G	G/G	G/G	G/A
62	BH	1	62	IDD	G/G	A/G	A/A	A/G	G/C	G/G	G/G
63	BH	1	63								

Fig. 6A

						Bahrein patients					
sample id	key	plate	original name	subgroup	TNFa (-308)	LT (249)	ESE3 (-140)	CTLA4 (+49)	TGFb (25)	Il-6P	ESE3 (+4458)
64	BH	1	64	NIDD	G/G	A/A	A/A		G/C		
65	BH	1	65	NIDD	G/G	A/A	A/A	A/A	G/G	G/C	G/G
66	BH	1	66	NIDD	G/G	A/A	A/A	A/G	G/G	G/C	A/A
67	BH	1	67	NIDD	G/G	A/G	A/A	A/A	G/G	G/G	G/G
68	BH	1	68	NIDD	G/G	A/A	A/A	A/G	G/G	G/G	G/G
69	BH	1	69	NIDD	G/G	A/G		A/G	G/G	C/C	G/G
70	BH	1	70	NIDD	G/G	A/A	A/G	A/G	G/C	G/G	G/A
71	BH	1	71	NIDD		A/G			G/G		G/A
72	BH	1	72	NIDD	G/G	A/G	A/G	A/A	G/C	G/G	G/G
73	BH	1	73	NIDD	G/G	A/A	A/A	G/G	G/G	G/G	A/A
74	BH	1	74	NIDD	G/G	A/A	A/A	A/A	G/G	G/G	A/A
75	BH	1	75	NIDD	G/A	A/G	A/A	A/G	G/G	G/G	G/G
76	BH	1	76	NIDD	G/G	A/G	A/A	A/A	G/G	G/C	G/A
77	BH	1	77	NIDD	G/G	A/G	A/G	A/A	G/G	G/G	G/A
78	BH	1	78	NIDD	G/G	A/A	G/G	A/G	G/G	G/G	G/G
79	BH	1	79	NIDD	G/G	A/G	A/A	A/G	G/G	G/G	G/G
80	BH	1	80	NIDD	G/A	A/G	A/G	A/A	G/G	G/G	G/A
81	BH	1	81	NIDD	G/G	A/A	A/A	A/G	G/G		G/A
82	BH	1	82	NIDD	G/G	A/A	A/A	A/A	G/G		G/A
83	BH	1	83	NIDD	G/G	A/A	A/A	A/G	G/G		G/G
84	BH	1	84	NIDD	G/G	A/G	A/G	A/A	G/G		A/A
85	BH	1	85	NIDD	G/G	A/A	A/A	A/G	G/G		G/G
86	BH	1	86	NIDD	G/G	A/A	A/A	A/A	G/C		G/G
87	BH	1	87	NIDD	G/G	A/A	A/A	A/A	G/G		G/G
88	BH	1	88	NIDD	G/G	G/G	A/A	A/G	G/G		G/G
89	BH	1	89	NIDD	G/G	A/A	A/A	A/G	G/G		G/G
90	BH	1	90	NIDD	G/G	A/A	A/A	A/A	G/G		G/G
91	BH	1	91	NIDD	G/G	A/G	A/A	A/A	G/G		G/G
92	BH	1	92	NIDD	G/G	A/G	A/G	A/A	G/G		A/A
93	BH	1	93	NIDD	G/G	G/G	A/G	A/A	G/G		G/A
94	BH	1	94	NIDD	G/G	A/G	A/A	A/G	G/G		G/A
95	BH	1	95	NIDD	G/G	A/A	A/A	A/G	G/G		
96	BH	1	96	NIDD	G/G	A/G	A/A	G/G	G/G		
97	BH	2	97	NIDD	G/G	A/G	A/A	A/A	G/G	G/C	
98	BH	2	98	NIDD	G/G	A/G	A/G	A/A		G/C	
99	BH	2	99	NIDD	G/G	A/G	A/A	A/A	G/G	G/G	
100	BH	2	100	NIDD	G/A	G/G	A/A	A/G	G/G	G/G	
101	BH	2	101	NIDD	G/A	A/G	A/A	A/G	G/G	G/G	
102	BH	2	102	NIDD	G/G	A/A	A/G	A/G	G/G	G/G	
103	BH	2	103	control	G/G	A/A	A/A	A/A	G/G	G/G	
104	BH	2	104	control	G/G	A/A	A/A	A/A	G/G	G/C	
105	BH	2	105	control	G/G	A/A	A/A	A/G	G/G		
106	BH	2	106	control	G/A	G/G	A/A	A/G	G/G	G/G	
107	BH	2	107	control	G/G	A/A	A/A	A/A	G/G	G/G	
108	BH	2	108	control	G/G	A/A	A/A	A/G	G/G	G/G	
109	BH	2	109	control	G/G	A/G	A/A	A/G	G/G	G/G	
110	BH	2	110	control	G/G	A/A	A/A	A/G	G/G	G/G	
111	BH	2	111	control	A/A	G/G	A/A	A/A	G/G	G/G	
112	BH	2	112	control	G/G	A/G	G/G	A/G	G/G	G/G	
113	BH	2	113	control	G/A	A/G	A/A	A/G	G/G		
114	BH	2	114	control	G/G	A/A	A/A	A/A	G/C	G/C	
115	BH	2	115	control	G/G	A/A	A/A	A/G	G/G	G/G	
116	BH	2	116	control	G/G	A/A	G/G	A/A	G/G	G/C	
117	BH	2	117	control	G/G	G/G	A/A	A/A	G/G	G/G	
118	BH	2	118	control	G/G	G/G	A/G	A/A	G/G	G/C	
119	BH	2	119	control	G/G	A/G	A/A	A/A	G/G	G/C	
120	BH	2	120	control	G/G	A/A	A/G	A/A	G/C	G/G	
121	BH	2	121	control	G/G	A/A	A/A	A/G	G/G	G/G	
122	BH	2	122	control	G/G	A/A	A/A	A/A	G/G	G/G	
123	BH	2	123	control	G/G	A/G	A/A	A/G	G/C	G/G	
124	BH	2	124	control	G/A	A/G	A/G	A/A	G/G	G/G	
125	BH	2	125	control	G/G	A/A	A/A	A/A	G/C	G/G	
126	BH	2	126	control	G/A	A/G	A/A	A/A	G/G	G/C	

Fig. 6B

						Bahrein patients					
sample id	key	plate	original name	subgroup	TNFa (-308)	LT (249)	ESE3 (-140)	CTLA4 (+49)	TGFb (25)	Il-6P	ESE3 (-4458)
127	BH	2	127	control	G/G	A/A	A/A	A/A	G/G	G/G	
128	BH	2	128	control	G/G	A/A	A/G	A/G	G/G	G/G	
129	BH	2	129	control	G/G	A/G	A/A	A/G	G/C	G/G	
130	BH	2	130	control	G/G	A/A	A/A	A/A	G/G	G/G	
131	BH	2	131	control	G/G	A/G	A/A	A/G	G/G	G/G	
132	BH	2	132	control	G/G	A/A	A/A	A/A	G/G	G/C	
133	BH	2	133	control	G/A	A/G	A/G		G/G		
134	BH	2	134	control	G/G	A/A	A/A	A/A	G/C	G/G	
135	BH	2	135	control	G/G	A/A	A/G	A/G	G/G	G/G	
136	BH	2	136	control	G/A	G/G	A/A	A/A	G/C	G/C	
137	BH	2	137	control	G/G	A/G	A/A	A/G	G/G		
138	BH	2	138	control	G/G	A/A	A/A	A/G	G/G	G/G	
139	BH	2	139	control	G/G	A/G	A/G	A/G	G/G	G/G	
140	BH	2	140	control	G/G	A/G	A/G	A/G	G/G	G/G	
141	BH	2	141	control	G/A	A/G	A/A	A/A	G/G	G/G	
142	BH	2	142	control	G/G	A/A	A/G	A/G	G/G	G/G	
143	BH	2	143	control	G/G	A/G	A/G	A/G	G/G	G/C	
144	BH	2	144	control	G/G	A/A	A/A	A/G	G/C	G/G	
145	BH	2	145	control	G/G	A/A	A/A	A/A	G/G	G/G	
146	BH	2	146	control	G/G	A/A	A/G	A/G	G/G	G/C	
147	BH	2	147	control	A/A	G/G	A/A	A/A	G/G	G/G	
148	BH	2	148	control	G/G	A/G	A/A	A/A	G/G	G/G	
149	BH	2	149	control	G/G	A/G		A/G	G/G	G/G	
150	BH	2	150	control	G/G	A/A	A/A	A/G	G/G	G/C	
151	BH	3	I-1	IDD	G/G	A/A	A/A	G/G		G/G	G/G
152	BH	3	I-2	IDD	G/G	A/G	A/A			G/G	
153	BH	3	I-3	IDD	G/G	A/G	A/A	A/A		G/G	G/A
154	BH	3	I-4	IDD	G/G		A/A	A/A			
155	BH	3	I-5	IDD	G/G	G/G	A/A	A/A		G/G	G/G
156	BH	3	I-6	IDD	G/G	G/G	A/A		G/G	G/G	G/G
157	BH	3	I-7	IDD	G/G	A/G	A/A	A/G		G/G	G/A
158	BH	3	I-8	IDD	G/A	A/G		G/G	G/G		G/A
159	BH	3	I-9	IDD		A/G	A/G	A/G	G/G		G/A
160	BH	3	I-10	IDD	G/G	A/G	A/G	A/A	G/G	G/G	G/G
161	BH	3	I-11	IDD	G/G	A/G	A/A	A/A	G/G	G/C	G/A
162	BH	3	I-12	IDD	G/G	A/A	A/A	A/G	G/C	G/G	G/G
163	BH	3	I-13	IDD	G/A	A/A	A/A		G/G	G/G	G/G
164	BH	3	I-14	IDD	G/G	A/G		A/G	G/C		G/G
165	BH	3	I-15	IDD	G/A	G/G	A/A	A/A	G/G	G/C	G/G
166	BH	3	I-16	IDD	G/G	A/G	A/G	A/G	G/G	G/G	G/G
167	BH	3	I-17	IDD	G/G	A/G	A/A	A/G	G/G		G/G
168	BH	3	I-18	IDD	G/G	A/G	A/G	A/G	G/G		G/G
169	BH	3	I-19	IDD	G/G	A/A	A/A	A/A	G/C	G/G	G/G
170	BH	3	I-20	IDD	G/G	A/G	A/G	A/G	G/G	G/G	G/G
171	BH	3	I-21	IDD	G/G	A/A	A/G	A/A	G/G	G/G	G/A
172	BH	3	I-22	IDD	A/A	G/G	A/G	A/G	G/G	G/G	G/G
173	BH	3	I-23	IDD	G/A	A/G	A/G	A/G	G/G	G/G	G/A
174	BH	3	I-24	IDD	G/A	G/G	A/G	A/A	G/G	G/G	
175	BH	3	I-25	IDD		G/G					
176	BH	3	I-26	IDD	G/A		A/G	A/A	G/G	G/C	
177	BH	3	I-27	IDD	G/G	A/A	A/G	A/A	G/G	G/G	
178	BH	3	I-28	IDD		A/A	A/G	A/A		G/G	
179	BH	3	I-29	IDD	G/G	G/G	A/G	A/G	G/G		
180	BH	3	I-30	IDD	G/G	G/G	A/A	A/A	G/G	G/C	G/G
181	BH	3	I-31	IDD	G/A	A/G	A/A	A/A	G/G	G/G	G/A
182	BH	3	I-32	IDD	G/G	A/G	A/G	A/A	G/G	G/G	G/A
183	BH	3	I-33	IDD	G/G	G/G			G/G	G/G	
184	BH	3	I-34	IDD	G/G	A/A		A/G	G/G	C/C	G/A
185	BH	3	I-35	IDD			A/G		G/G		
186	BH	3	I-36	IDD	G/G	A/A	A/A	A/A	G/G	G/C	G/G
187	BH	3	I-37	IDD	G/G	A/G	A/A	A/A	G/G	G/G	G/A
188	BH	3	I-38	IDD	G/A	A/G	A/A	A/G	G/G	G/G	G/A

Fig. 6C

						Bahrein patients					
sample id	key	plate	original name	subgroup	TNFa (-308)	LT (249)	ESE3 (-140)	CTLA4 (+49)	TGFb (25)	Il-6P	ESE3 (-4458)
189	BH	3	I-39	IDD	G/G	A/A		A/A	G/G	G/G	
190	BH	3	I-40	IDD	G/G	A/A	A/A		G/C		G/A
191	BH	3	I-41	IDD		A/A			G/C		
192	BH	3	I-42	IDD	G/G	A/A		A/G	G/G	G/G	
193	BH	3	NI-1	NIDD	G/A	A/G	A/G		G/G	G/G	G/G
194	BH	3	NI-2	NIDD	G/G	A/A	A/A	A/A	G/G	G/C	
195	BH	3	NI-3	NIDD	G/G	A/G	A/A	A/A	G/G	G/G	
196	BH	3	NI-4	NIDD	G/A	A/G	A/A	A/G	G/G		G/A
197	BH	3	NI-5	NIDD	A/A	G/G	A/A	A/A	G/G	G/C	G/G
198	BH	3	NI-6	NIDD		A/G	A/G	A/A		G/G	
199	BH	3	NI-7	NIDD		A/A		A/A			G/G
200	BH	3	NI-8	NIDD	G/G	A/G	A/G	A/G	G/G	G/C	G/A
201	BH	3	NI-9	NIDD	G/G	A/A	A/A	A/A	G/G	G/G	G/A
202	BH	3	NI-10	NIDD	G/A	A/G	A/A	A/G	G/G	G/G	G/A
203	BH	3	NI-11	NIDD	G/G	A/A	A/G	A/G	G/C	G/G	G/G
204	BH	3	NI-12	NIDD	G/G	A/A	A/A	A/A	G/G	G/G	G/G
205	BH	3	NI-13	NIDD	G/G	A/A	A/A	A/A	G/G	G/C	G/A
206	BH	3	NI-14	NIDD	G/A	G/G	A/G	A/G	G/C		G/G
207	BH	3	NI-15	NIDD		A/A		A/A	G/C		
208	BH	3	NI-16	NIDD	G/A	G/G	A/G	A/G	G/G	G/G	
209	BH	3	NI-17	NIDD	G/A	A/G	A/G	A/A	G/G	G/C	G/G
210	BH	3	NI-18	NIDD	G/G	A/G	A/A	A/A	G/G	G/G	G/G
211	BH	3	NI-19	NIDD		A/G		A/G	G/G	G/G	
212	BH	3	NI-20	NIDD	G/A	G/G	A/A	A/A	G/G	G/G	
213	BH	3	NI-21	NIDD	G/G	A/G	A/G	A/A	G/G	G/G	G/G
214	BH	3	NI-22	NIDD	G/A	A/G	A/A	A/A	G/G	G/G	G/G
215	BH	3	NI-23	NIDD	A/A	A/A	A/G	A/A	G/G	G/G	G/G
216	BH	3	NI-24	NIDD	G/G	A/G		A/A	G/G	G/C	G/G
217	BH	3	NI-25	NIDD	G/A	A/G	A/G	G/G	G/G	G/G	G/A
218	BH	3	NI-26	NIDD	G/G	A/A		A/A	G/G	G/C	G/G
219	BH	3	NI-27	NIDD		A/A	A/G	A/A		G/G	G/A
220	BH	3	NI-28	NIDD	G/G	A/A	A/G	A/A	G/G	G/G	G/A
221	BH	3	NI-29	NIDD	G/A	A/G	A/G	A/A	G/G	G/G	G/G
222	BH	3	NI-30	NIDD	G/G	A/A		A/A		G/G	G/G
223	BH	3	NI-31	NIDD	G/G	A/A	A/A	A/A	G/G	G/G	G/A
224	BH	3	NI-32	NIDD	G/G	A/A	A/A	A/A	G/G	G/G	G/G
225	BH	3	NI-33	NIDD	G/G	A/A	A/G	A/G	G/G	G/G	G/G
226	BH	3	NI-34	NIDD	G/G	A/G		A/G	G/G	G/C	G/A
227	BH	3	NI-35	NIDD	G/G	A/A	A/A	A/A	G/G	G/G	G/G
228	BH	3	NI-36	NIDD	G/G	A/G	A/A	A/A	G/C	G/G	G/G
229	BH	3	CO-1	control	G/G	A/A	A/A	A/A	G/G	G/C	G/G
230	BH	3	CO-2	control	G/G	A/A	A/G	A/A	G/G	G/G	
231	BH	3	CO-3	control	G/G	G/G	A/A	A/A	G/G	G/G	G/G
232	BH	3	CO-4	control	G/G	A/G	A/A	A/A	G/G	G/G	G/G
233	BH	3	CO-5	control				A/A	G/C		
234	BH	3	CO-6	control	G/G	A/G	A/G	A/A	G/G		G/G
235	BH	3	CO-7	control		A/G		A/A	G/G	G/G	G/G
236	BH	3	CO-8	control	G/A					G/G	
237	BH	3	CO-9	control	G/G	A/G	A/A	A/G	G/G	G/G	G/G
238	BH	3	CO-10	control		A/G		A/G			G/A
239	BH	3	CO-11	control		A/G		A/A	G/G	G/G	
240	BH	3	CO-12	control	G/A	A/G	A/G	A/A	G/G	G/G	G/G
241	BH	3	CO-13	control	G/A	A/A	A/A	A/A	G/G	G/G	G/G
242	BH	3	CO-14	control	G/A	A/G	A/A	A/A	G/G	G/C	G/G
243	BH	3	CO-15	control	G/A	A/G	A/A	A/A	G/G	G/C	G/G
244	BH	3	CO-16	control	G/A	A/A	A/G	A/A	G/G	G/G	G/G
245	BH	3	CO-17	control		A/A		A/G	G/G	G/G	G/G
246	BH	3	CO-18	control		A/A	A/A	A/A	G/G	G/G	G/G
247	BH	4	CO-19	control		A/A	A/A	A/A	G/G	G/G	G/A
248	BH	4	CO-20	control		A/G	A/G	A/A	G/G	G/G	G/A
249	BH	4	CO-21	control	G/G	G/G	A/A	A/G	G/G	G/G	G/A
250	BH	4	CO-22	control	G/G	A/A	A/A	A/A	G/G	G/G	G/G

Fig. 6D

						Bahrein patients					
sample id	key	plate	original name	subgroup	TNFa (-308)	LT (249)	ESE3 (-140)	CTLA4 (+49)	TGFb (25)	Il-6P	ESE3 (-4458)
251	BH	4	CO-23	control	G/A	A/G	A/A	A/A	G/C		
252	BH	4	CO-24	control	G/G	A/G	A/A	A/A		G/G	G/G
253	BH	4	CO-25	control	G/G	A/G	A/A	A/A	G/C	G/C	G/G
254	BH	4	CO-26	control	G/A	A/G	A/A	A/G	G/G	G/C	G/G
255	BH	4	CO-27	control	G/A	A/G	A/A	A/G		G/G	G/A
256	BH	4	CO-28	control	G/A	A/G	A/G	A/A	G/G	G/G	G/G
257	BH	4	CO-29	control		A/G	A/A	A/A		G/G	G/A
258	BH	4	CO-30	control	G/G	A/A	A/A	A/A	G/G	G/G	G/G
259	BH	4	CO-31	control		A/G	A/G		G/G	G/C	G/G
260	BH	4	CO-32	control	G/G	G/G	A/A			G/G	
261	BH	4	CO-33	control	G/G	G/G	A/A		G/G		
262	BH	4	CO-34	control	G/G	A/A	A/A	A/A	G/G	G/C	G/G
263	BH	4	CO-35	control			A/A			G/G	G/A
264	BH	4	CO-36	control	G/G	A/A	A/A	A/A	G/G	G/G	G/A
265	BH	4	CO-37	control	G/G		A/A			G/C	G/G
266	BH	4	CO-38	control	G/G	A/A	A/G	A/A	G/G		G/A
267	BH	4	CO-39	control	G/G	A/A	A/A			G/G	
268	BH	4	CO-40	control		A/A	A/A		C/C	G/G	
269	BH	4	CO-41	control	G/G	A/G	A/A	A/A	G/G		G/A
270	BH	4	CO-42	control		A/G	A/A	A/G			G/G
271	BH	4	CO-43	control	G/G	A/G	A/G	A/G	G/G	G/G	G/G
272	BH	4	CO-44	control	G/A	A/G	A/A	A/G	G/G		G/G
273	BH	4	CO-45	control			A/A			G/G	
274	BH	4	CO-46	control	G/G	A/G	A/A	A/G	G/G		G/G
275	BH	4	CO-47	control		A/A	A/G			C/C	A/A
276	BH	4	CO-48	control	G/G	A/G	A/G		G/C	G/G	G/G

Fig. 6E

Bahrein Patients

		TNF (-308)		LT (249)		ESE3 (-140)	
IDD	wt/wt	74	75%	36	36%	53	56%
	wt/SNP	22	22%	42	42%	41	43%
	SNP/SNP	3	3%	23	23%	1	1%
		99		101		95	
NIDD	wt/wt	53	77%	34	45%	42	62%
	wt/SNP	14	20%	34	45%	25	37%
	SNP/SNP	2	3%	7	10%	1	1%
		69		75		68	
Controls	wt/wt	61	75%	40	44%	65	73%
	wt/SNP	18	22%	41	45%	22	25%
	SNP/SNP	2	3%	10	11%	2	2%
		81		91		89	

Bahrein Patients

		CTLA4 (+49)		TGFβ (25)		IL-6P		ESE-3 (-4458)	
IDD	wt/wt	47	50%	80	83%	65	74.0%	59	67%
	wt/SNP	44	46%	16	17%	22	25.0%	27	31%
	SNP/SNP	4	4%	0		1	1.0%	2	2%
		95		96		88		88	
NIDD	wt/wt	43	61%	62	89%	39	73.5%	35	59%
	wt/SNP	25	35%	8	11%	13	24.5%	19	32%
	SNP/SNP	3	4%	0		1	1.8%	5	9%
		71		70		53		59	
Controls	wt/wt	50	60%	71	84%	62	76.0%	27	71%
	wt/SNP	34	40%	13	15%	19	23.0%	10	26%
	SNP/SNP	0		1	1%	1	1.0%	1	3%
		84		85		82		38	

Fig. 7

Population: BH	Cols: phenotype	Rows: TNFa_n308	
Observed	control	IDD	NIDD
MUT	20	25	16
WT	61	74	53
DF:	2	X2:	9.61E-02

Expected	control	IDD	NIDD
MUT	19.84337	24.25301	16.90361
WT	61.15663	74.74699	52.09639
p=	0.953086		

Population: BH	Cols: phenotype	Rows: LT_249	
Observed	control	IDD	NIDD
AA	40	36	34
AG	41	42	34
GG	10	23	7
DF:	4	X2:	8.079073

Expected	control	IDD	NIDD
AA	37.49064	41.61049	30.89888
AG	39.8764	44.25843	32.86517
GG	13.63296	15.13109	11.23596
p=	0.088724		

Population: BH	Cols: phenotype	Rows: ESE3_n140	
Observed	control	IDD	NIDD
MUT	24	42	24
WT	65	53	42
DF:	2	X2:	5.935781

Expected	control	IDD	NIDD
MUT	32.04	34.2	23.76
WT	56.96	60.8	42.24
p=	0.051412		

Population: BH	Cols: phenotype	Rows: CTLA4_49	
Observed	control	IDD	NIDD
MUT	34	48	29
WT	50	47	43
DF:	2	X2:	2.462644

Expected	control	IDD	NIDD
MUT	37.14741	42.01195	31.84064
WT	46.85259	52.98805	40.15936
p=	0.291906		

Fig. 8

Sample	PCR #	Annotation	Patient	Phenotype ESE3(-140)	Annotation	Disease
1	1	AH	MS	A/G	AH	MS
2	2 and 63	BG	control	A/G	BG	control
3	3	BM	MS	A/G	BM	MS
4	4	BN	MS	A/A	BN	MS RR
5	5	CM	MS	A/G	CM	MS
6	6	CT	MS	A/G	CT	MS
7	7	DS	MS	A/A	DS	MS
8	8	GR	MS	A/A	GR	MS 2P
9	9	HH	MS	A/G	HH	MS RR
10	10	HK-1	MS	A/G	HK1	MS
11	11	HK-2	MS	A/A	HK2	MS RR
12	12	JHR	control	A/G	JHR	control
13	13	KT	MS	A/G	KT	MS RR
14	14	LC	MS	A/G	LC	MS RR
15	15	LFA	MS	A/G	LFA	MS RR
16	16	LLK	MS	A/A	LLK	MS RR
17	17	MV	MS	A/A	MV	MS
18	18	NE	MS	A/A	NE	MS RR
19	19	OE	control	A/A	OE	control
20	20	PR	MS	A/A	PR	MS
21	21	SD	MS	A/G	SD	MS RR
22	22	SR	MS	A/G	SR	MS RR
23	23	TR	MS	A/G	TR	MS 1P
24	24	VR	MS	A/A	VR	MS RR
25	25	WB	MS	A/G	WB	MS
26	26	WC	MS	A/G	WC	MS RR
27	27	WE	control	A/A	WE	control
28	28	AJ	IDDM	A/A	AJ	IDDM
29	29	DC	IDDM	A/G	DC	IDDM
30	30 / 62	EA	control	A/A	EA	control
31	31	EJ	control	A/A	EJ	control
32	32	FJ	IDDM	A/A	FJ	IDDM
33	33	FL	control	A/A	FL	control
34	34	GC	IDDM	A/G	GC	IDDM
35	35 / 67	GN	control	A/A	GN	control
36	36	MA	IDDM	A/A	MA	IDDM
37	37	RD	IDDM	A/A	RD	IDDM
38	38	WM	IDDM	A/A	WM	IDDM
39	39	YP	IDDM	A/A	YP	IDDM
40	40	BJ	control	A/A	BJ	control
41	41	BAC	control	A/A	BAC	control
42	42	CA	control	A/A	CA	control
43	43	DA	control	A/A	DA	control
44	44	DF	control	A/A	DF	control
45	45	DN	control	A/A	DN	control
46	46	IZ	control	A/A	IZ	control
47	47	KD	control	A/A	KD	control
48	48	OCK	control	A/A	OCK	control
49	49	RK	control	A/A	RK	control
50	50	SJ	control	A/G	SJ	control
51	1	CB 57	MS	A/G	CB57	MS RR
52	2	DB 53	MS	A/A	DB53	MS 2P
53	3	EB 77	MS	A/G	EB77	MS RR

Fig. 9A

54	4	GB 59	MS	A/A	GB59	MS RR
55	5	JB 43	MS	A/G	JB43	MS 2P
56	6	RB 46	MS	A/A	RB46	MS RR
57	7	TB 77	MS	A/A	TB77	MS RR
58	8	BC 61	MS	A/A	BC61	MS RR
59	9	JC 53	MS	A/A	JC53	MS RR
60	10	MC 74	MS	A/G	MC74	MS RR
61	11	SC 59	MS	A/A	SC59	MS RR
62	12	JD 37	MS	A/A	JD37	MS 2P
63	13	KD 49	MS	A/A	KD49	MS 2P
64	14	MD 57	MS	A/A	MD57	MS 2P
65	15	RD 48	MS	A/A	RD48	MS 2P
66	16	TD 56	MS		TD56	MS RR
67	17	EG 60	MS	A/A	EG60	MS RR
68	18	LG 71	MS	A/A	LG71	MS 1P
69	19	LG 45	MS	A/A	LG45	MS 2P
70	20	RG 55	MS	A/G	RG55	MS 2P
71	21	RG 56	MS	A/A	RG56	MS
72	22	TG 54	MS	A/A	TG54	MS 2P
73	23	BH 57	MS	A/A	BH57	MS 2P
74	24	DCH 43	MS	A/A	DCH43	MS 2P
75	25	SH 50	MS	A/G	SH50	MS RR
76	26	AJ 66	MS	A/A	AJ66	MS 2P
77	27	CK 60	MS	A/G	CK60	MS RR
78	28	MJ 80	MS	A/G	MJ80	MS RR
79	29	DK 44	MS	A/A	DK44	MS 1P
80	30	SK 40	MS	A/A	SK40	MS 2P
81	31	LL 65	MS	A/A	LL65	MS RR
82	32	BN 48	MS	A/G	BN48	MS RR
83	33	KN 65	MS	A/G	KN65	MS RR
84	34	JO 54	MS	A/A	JO54	MS RR
85	35	MO 50	MS	A/A	MO50	MS RR
86	36	VO 69	MS	A/A	VO69	MS RR
87	37	MP 63	MS	A/A	MP63	MS RR
88	38	RP 58	MS	A/A	RP58	MS 1P
89	39	KR 66	MS	A/G	KR66	MS RR
90	40	SFR 56	MS	A/G	SFR56	MS RR
91	41	FS 58	MS	G/G	FS58	MS 1P
92	42	JS 32	MS	A/A	JS32	MS 2P
93	43	MS 47	MS	A/A	MS47	MS 2P
94	44	PS 55	MS	A/A	PS55	MS RR
95	45	SS 49	MS	A/A	SS49	MS 2P
96	46	SS 58	MS	A/A	SS58	MS 2P
97	47	SS 62	MS	A/G	SS62	MS RR
98	49	BV 63	MS	A/A	BV63	MS RR
99	50	SV 55	MS	A/A	SV55	MS RR
100	51	CW 68	MS	A/G	CW68	MS RR
101	52	LW 57	MS	A/A	LW57	MS RR
102	53	TW 62	MS	A/A	TW62	MS 2P
103	54	DY 45	MS	A/G	DY45	MS 2P
104	70	11 GF	MS	A/A	11.GF	MS
105	71	1. GF	MS	A/G	1.GP	MS 2P
106	72	2. GF	MS	A/G	2.GF	MS 1P
107	73	3. GF	MS	A/A	3.GF	MS RR
108	74	4. GF	MS	A/A	4.GF	MS RR

Fig. 9B

109	75	5. GF	MS	A/A	5.GF	MS RR
110	76	6. GF	MS	A/G	6.GF	MS RR
111	77	7. GF	MS	A/G	7.GF	MS RR
112	78	8.GF	MS	A/G	8.Kasia	
113	79	9. GF	MS	A/A	9.GF	MS 2P
114	80	10. GF	MS	A/G	10.GF	MS RR
115	55	ABO 63	control	A/G	ABO63	control
116	56	AS 69	control	A/A	AS69	control
117	58	JH spose	control	A/G	JH spouse	control
118	59	MM 57	control	A/A	MM57	control
119	60	RT 22 spose	control	A/A	RT spouse	control
120	61	TT	control	A/G	TT	control
121	64	ZV	control	A/A	ZV	control
122	65	GJ	control	A/A	GJ	control
123	66	DG	control	A/G	DG	control
124	68	GF mother	control	A/A	GF mother	control
125	69	GF father	control	A/A	GF father	control
					3k GF	control
					12.GF	MS
	48	RT 22	Alzheimer	G/G	13. GF	MS
					14.GF	MS RR
					15.GF	MS RR
					16.GF	MS
					SA-S	IDDM (Sally)
					BC	(my)control
					CB-S	IDDM (Sally)
					KA	(my)control
					17.GF	MS
					18.GF	MS
					DH	(my)control
					MA	control
					ODS-V	IDDM (Vissia)
					GrB-V	control (Vissia)

Fig. 9C

ESE3 (-140)

	MS		controls		IDDM	
WT/WT	50	58%	23	76%	6	75%
WT/SNP	35	41%	7	24%	2	25%
SNP/SNP	1	1%	0		0	
	86		30		8	

Fig. 10

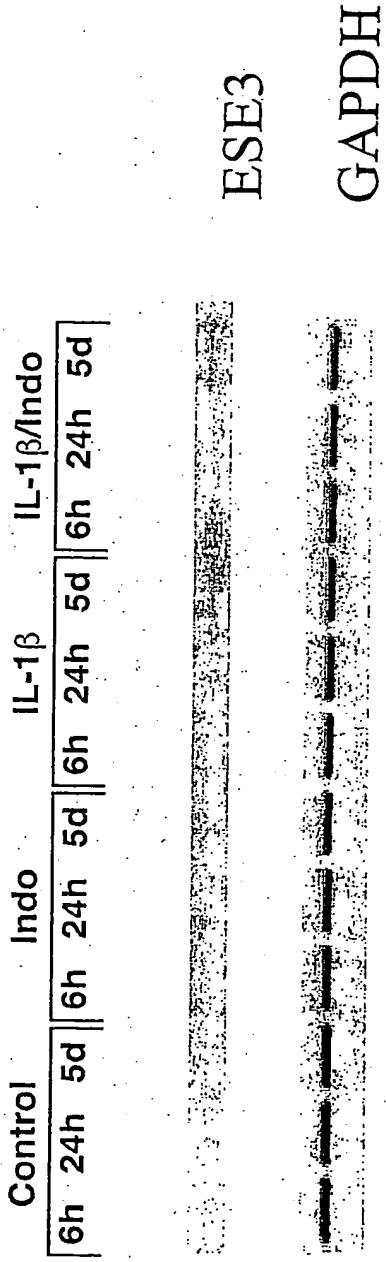

ESE3
GAPDH
Control
Indo
IL-1β
IL-1β/Indo
6h 24h 5d 6h 24h 5d 6h 24h 5d 6h 24h 5d

Fig. 11A

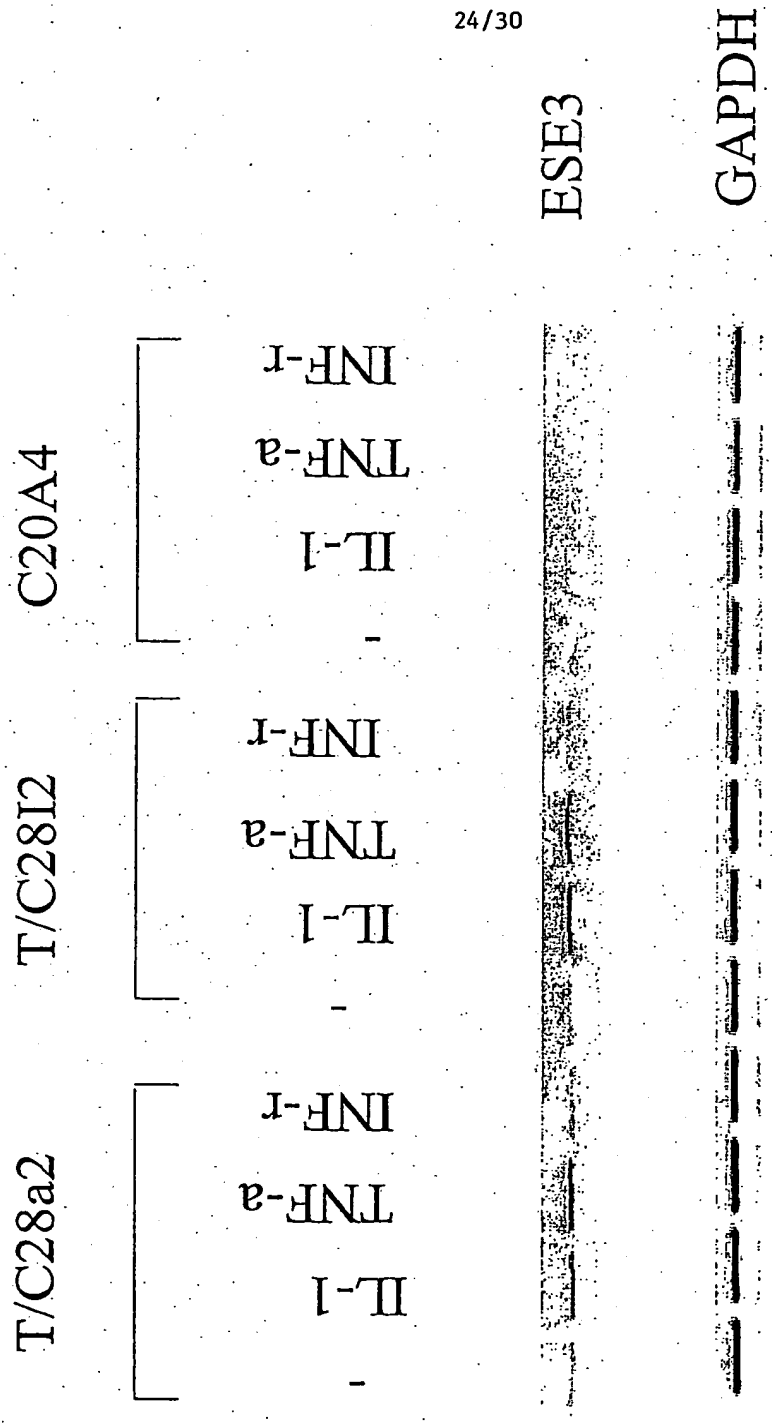


Fig. 11B

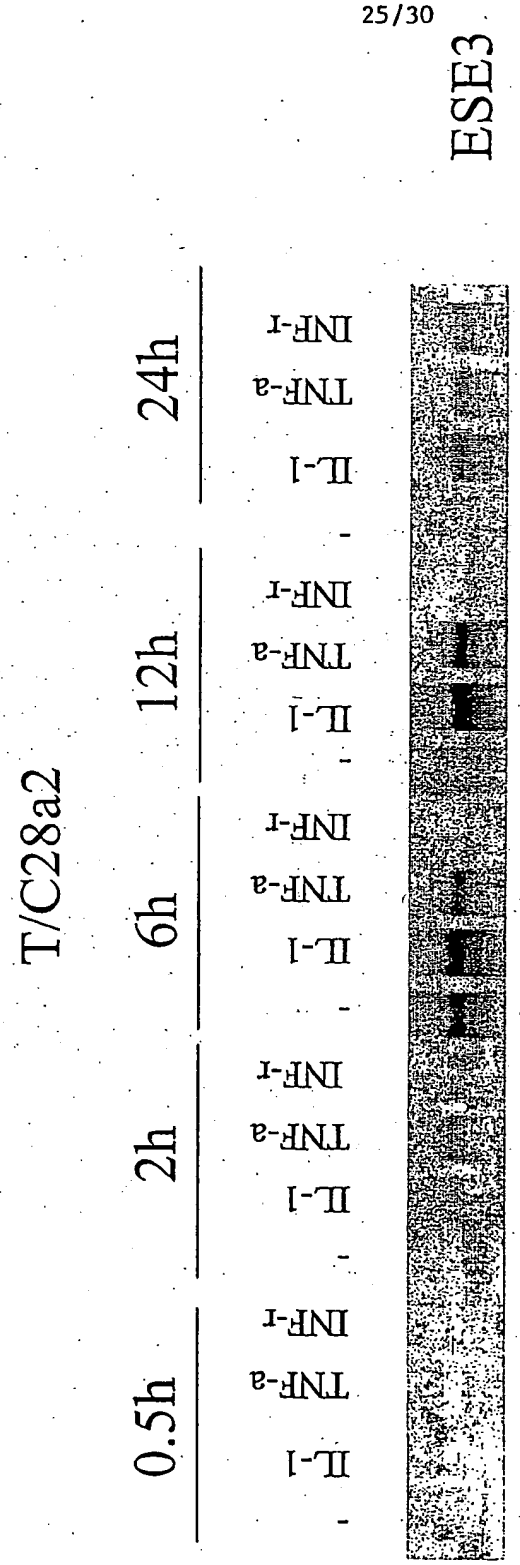


Fig. 11C

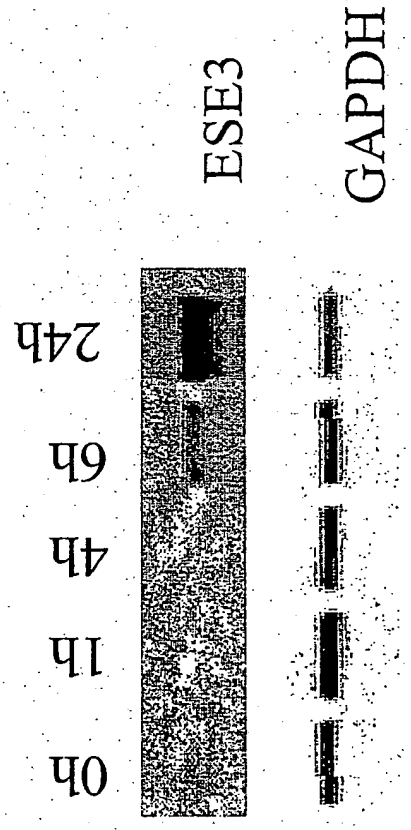


Fig. 11D

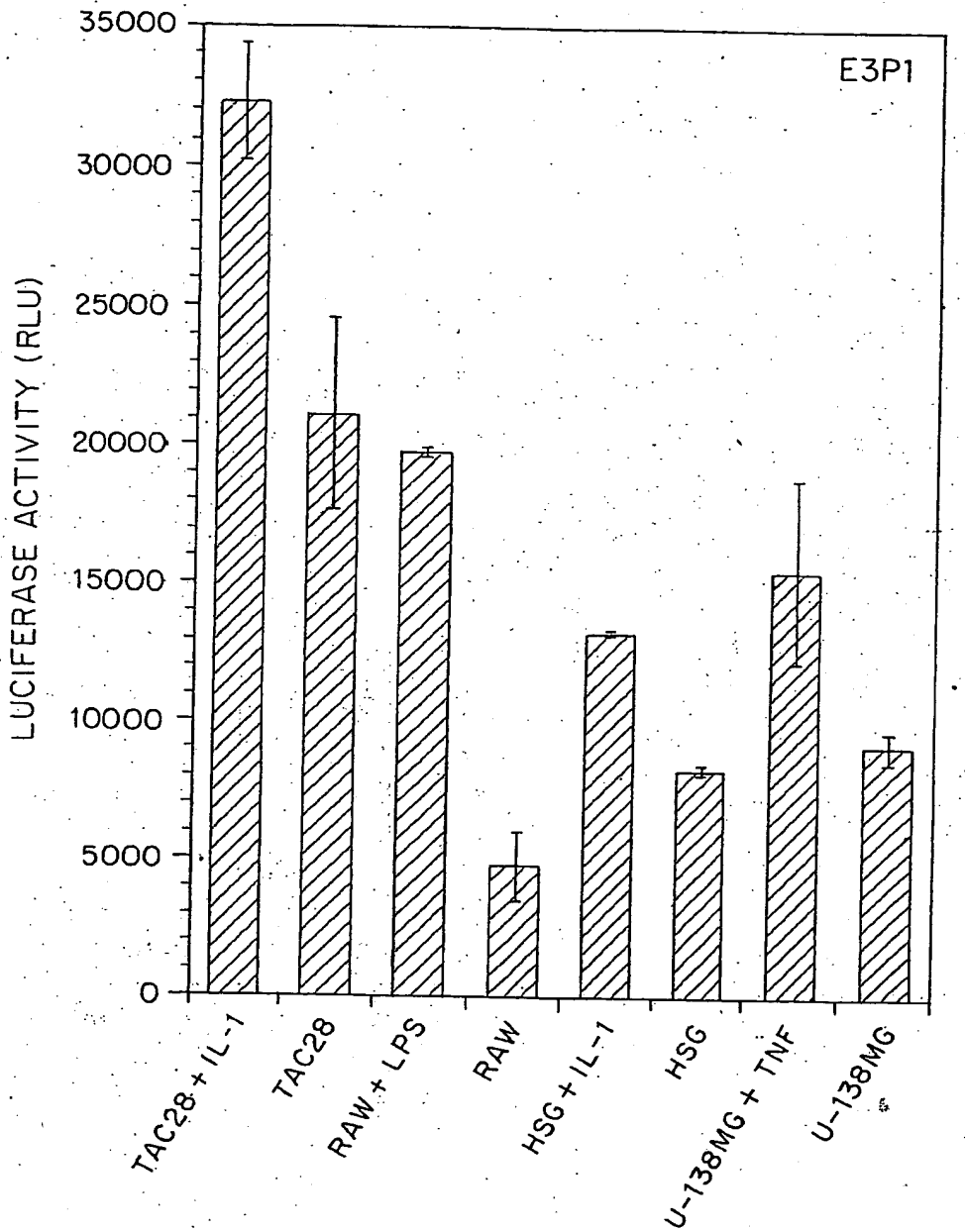

E3P1
LUCIFERASE ACTIVITY (RLU)
35000
30000
25000
20000
15000
10000
5000
0
TAC28 + IL-1
TAC28
RAW + LPS
RAW
HSG + IL-1
HSG
U-138MG + TNF
U-138MG

FIG. 12A

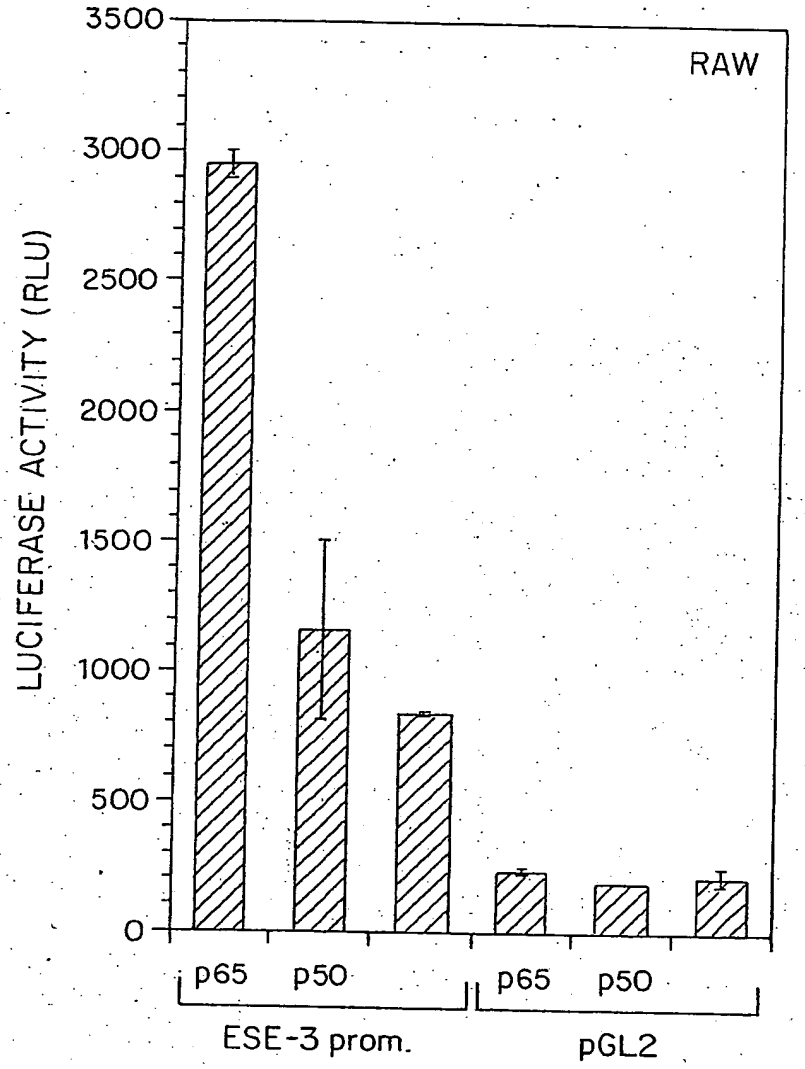


FIG. 12B

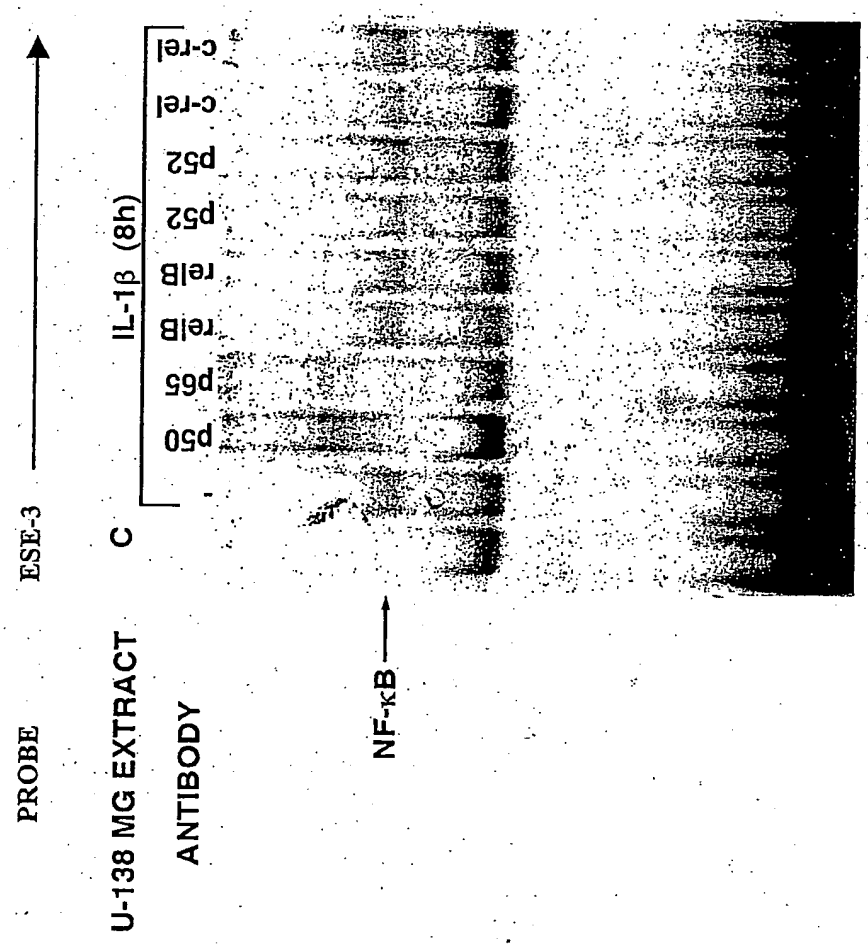

PROBE
ESE-3
U-138 MG EXTRACT
C
IL-1β (8h)
ANTIBODY
p50
p65
relB
relB
p52
p52
c-rel
c-rel
NF-κB

Fig. 13

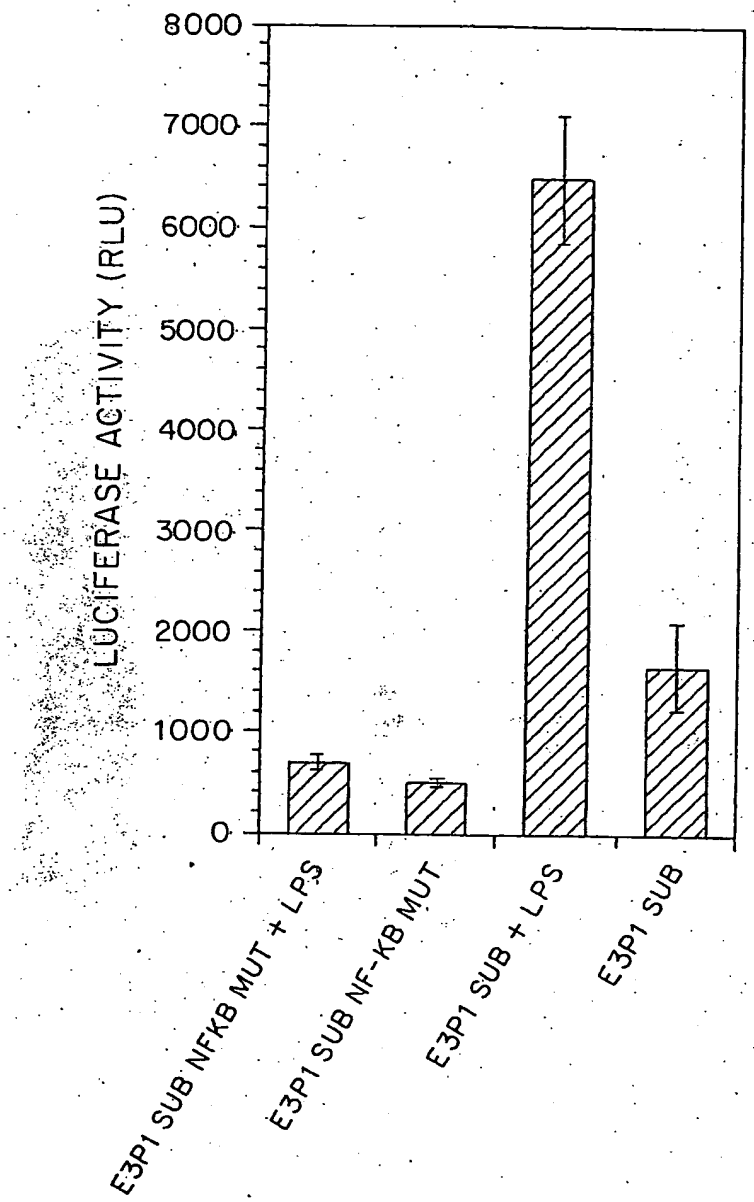


FIG. 14

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS OF DIAGNOSIS OF AUTOIMMUNE DISEASE

(57) Abstract: Methods of diagnosis of autoimmune disease or of a predisposition or susceptibility to autoimmune disease by detecting a polymorphism in the ESE-1, ESE-2 or ESE-3 gene. Methods of therapy of autoimmune disease in individuals having a polymorphism in the ESE-1, ESE-2 or ESE-3 gene.

WO 2003/034896 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/32116

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12Q 1/68; A61K 31/70
US CL : 436/6; 514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 436/6; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST 2.0, MEDLINE, CAPLUS, EMBASE, BIOTECH, BIOSIS, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BREMBECK, F et al. Dual function of the epithelial specific ets transcription factor, ELF3, in modulating differentiation. Oncogene. January 2000, Vol. 19, pages 1941-1949, see entire reference.	1-31, 33-40
A	WO 99/55728 A1 (HSC RESEARCH AND DEVELOPMENT LIMITED PARTNERSHIP) 04 November 1999 (04.11.1999), see entire patent.	1-31, 33-40
A	WO 99/65929 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 23 December 1999 (23.12.1999), see entire patent.	1-3, 33-40

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search
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Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/32116

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claim Nos.: 32
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.